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The Metabolism of Chlorella.

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These tests were carried out using the following species of Chlorella: *C. variegatus, viscosa, vulgaris var. genevensis, rubescens, luteo-viridis,* together with one specie not recognized. In addition *Chlorococcus humicola* and *Mannochloris bacillaris* were included within the series.

The base medium was Knopf's solution diluted 3 times with distilled water. When needed, it was rendered semisolid by 1.5% agar.

Nitrates are reduced to nitrites by 3 of these strains, C. sp?, *C. vulgaris,* and *C. rubescens* as indicated both by sulphanilic acid and by starch iodide. This reaction is slow but was definite in one month. On the other hand nitrite is oxidized to nitrate by *C. luteo-viridis* as proven by diphenylamine and again the test period was 30 days. Gelatine is liquefied very slowly by *C. rubescens.* Certain split products of protein are favorable to increased growth since peptone in amounts varying between 0.1% and 0.7% increase the growth of *C. vulgaris, C. rubescens,* and *C. luteo-viridis.* Urea in concentrations of 0.1% and 0.02% inhibits the growth of these 3 stains partially also, while ammonium carbonate in similar concentration was unfavorable to *C. rubescens* and *C. luteo-viridis* but stimulated slightly the growth of *C. vulgaris.*

A series of tests was made to determine the influence of amino acids upon the growth of these algae. This group of compounds included glutamic acid, valine, arginine, leucine, d- l- alanine, phenylalanine, histidine, aspartic acid, serin, tryptophane, glycine, tyrosine, cystine and cysteine. Isoelectric gelatine also was tested here. In comparison to controls, leucine increased the growth of *C. variegatus, Chlorococcus humicola, M. bacillaris,* C. sp.?, and *C. rubescens.* Glycine stimulated all of these algae as also did tyrosine, with the exception of C. sp?. Arginine aids proliferation of each alga with the exception of *M. bacillaris.* Alanine was beneficial to all but *Chlorococcus humicola* and *C. luteo-viridis.* Valin and serin were beneficial to all except *Chlorococcus humicola.* Phenylalanine stimulated *C. variegatus* and *C. rubescens* but in varying degree depressed the other cultures. With the exception of *Chlorococcus humicola* and C. sp.?, tryptophane gave greater growth than did the controls. Glutamic acid benefited *M. bacillaris* and *C. luteo-viridis* only. Aspartic acid was favorable to *M. bacillaris* and C. sp.?. Histidine and cysteine depressed all while cystine gave benefit with C. sp.?, *C. vulgaris,* and *C. rubescens.* Gelatine appears to be of value with *C. variegatus, M. bacillaris,* C. sp.?, and *C. rubescens.*

All cultures of these algae in which growth took place in the presence of amino acids became increasingly basic during the observation period of 6 weeks. All readings were made electrometrically. Control positives of inoculated media did not show this change. Control cultures grown without the addition of amino acids also showed a similar alteration but in much less degree. Shifts as great as 3.280 to 7.658 in the case of glutamic acid became evident. Control cultures without amino acids showed a shift from 5.302 to 6.808. Arginine media gave an initial reading of 8.199 and this in turn shifted to 8.301.

The effect of the following carbohydrates upon these algae was tested by the addition of 0.1% of the compound in question to the standard medium. This list included glucose, arabinose, potato starch, raffinose, maltose, xylose, inosite, inulin, salicin, levulose, melizitose, sucrose, rhamnose, dulcitol, trehalose, lactose and galactose together with control preparations. For *C. variegatus, Chlorococcus humicola, M. bacillaris,* C. sp.?, and *C. viscosa* two series of tests were made, the first by growth in diffuse daylight and the second in complete darkness. The growth period was 6 weeks at room temperature. All of these sugars with the exception of arabinose, inosite, and xylose stimulated the growth of *C. variegatus* in light but in the dark no stimulation was produced by the presence of

inosite, xylose, rhamnose, dulcitol, and lactose. Increased growth, however, resulted in darkness from the other carbohydrates tested.

All sugars with the exception of levulose and inulin increased the growth of *Chlorococcus humicola* in the light but at the same time in darkness the following sugars were the only ones to cause any growth whatever to appear: lactose rhamnose, xylose, sucrose, inosite, and trehalose. No growth appeared in the control flask in darkness.

The growth of *M. bacillaris* was stimulated in the light by all sugars except xylose while in the dark this organism was accelerated to growth by galactose, glucose, levulose, maltose, arabinose, lactose, inosite, sucrose, and starch. No growth under these conditions took place with any other sugar nor in the control flask.

C. sp.? in the light is not benefited and indeed is somewhat depressed by dulcitol, trehalose, inosite, salicin and xylose although in darkness every sugar in the series induced better growth than that in the control.

C. viscosa showed varying degrees of inhibition by arabinose, inosite, and xylose but the remainder of the series caused more luxuriant growth than that of the control flask. In the dark, however, no growth was evident with sucrose, melizitose, inulin, arabinose and xylose. Thus, inosite causes inhibition in diffuse light but in the dark it stimulates growth somewhat.

The following 3 forms were tested with carbohydrates only in the light. Growth of *C. vulgaris* was improved by the presence of maltose, raffinose, starch and levulose. Stimulation of growth of *C. rubescens* was induced by maltose, levulose, raffinose, salicin, galactose, dextrin and inosite while all sugars were beneficial to proliferation of *C. luteo-viridis.*

In nearly all instances electrometric measurements of these various cultures of algae whether grown in light or in darkness revealed a consistent decrease in hydrogen ion concentration as growth progressed. Thus, if acid be produced from sugars by the algal growth it is more than neutralized by basic by-products. In one instance when *C. viscosa* was grown with glucose this change was from 5.681 to 8.690 in 6 weeks. An inoculated portion of the same medium showed a negligible difference only during the same period.

As indicated by use of iodine, *C. variegatus* grown in the dark in the presence of levulose and melizitose elaborated starch within the cell. A similar effect was noted with *C. viscosa* in the presence of glucose.

4573

Cellulose Digestion by Organisms from the Termite Gut.

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The termite thrives in an environment which is most unusual and which requires specialized adaptation for survival. Its galleries are largely cellulose as also is its diet. Relatively little combined nitrogen is found in its food other than that derived from consumption of fecal materials of its fellows. That sector of the nitrogen cycle which includes the metabolism of the termite is as yet unexplored.

It is probable that the energy requirements of the termite are derived from the destruction of cellulose. In addition to the energy required by the animal for its ordinary life processes an additional large amount must be available should it develop that there is any fixation of nitrogen connected with its metabolism. The question of cellulose splitting within the gut of the termite thus is of fundamental importance.

Cellulose digestion is not unknown among the invertebrates. Miller and Boynton[1] have demonstrated the appearance of glucose within the gut of Bankia, the Northwest shipworm. Werner[2] has shown that the digestion of cellulose within the intestine of *Potosia cuprea,* the rose chafer, is bacterial in character. Cleveland,[3] however, believes that the enormous numbers of bacteria found within the gut of the termite have no function as cellulose splitters within the intestinal canal of *Reticulotermes flavipes,* and his series is reported to have included at least 100 individuals.

Inasmuch as opportunity presented itself, through the courtesy of Dr. S. F. Light, to obtain termites from a variety of sources and of a number of different genera, we decided to make a further attempt to demonstrate the splitting of cellulose by bacteria derived from termite gut content. The technique was that usually followed in soil bacteriology. Flasks were prepared containing medium of the following formula: K_2HPO_4—1 gm.; $MgSO_4$—1 gm.; Na_2CO_3—1 gm.; $(NH_4)_2SO_4$—2 gm.; $CaCO_3$—2 gm.; tap water 1000cc. A 2-inch circle of filter paper was added to each flask and the material then was autoclaved.

[1] Miller, R. C., and Boynton, L. C., *Science,* 1926, xliii, 524.

[2] Werner, E., *Cent. f. Bakt. u. Parasit.,* 1926, Abt. 2, lxvii, 297.

[3] Cleveland, L. R., *Biol. Bull.,* 1924, xlvi, 177.

Gut content was prepared by immersing the termite in tincture of iodine (U. S. P.) for 45 seconds. The animal afterwards was washed in each of 2 changes of sterile physiological saline. The gut was then exposed by use of fine forceps and with a sterile glass slide and its contents were placed as an inoculum within a flask of medium. Incubation took place under aerobic conditions at 20°-28° C. Evidence of cellulose digestion was offered as it occurred by disintegration of the filter paper. Control uninoculated flasks were placed with all series thus inoculated in order to make certain that mere physical disintegration was not concerned here.

Our series has included 85 flasks and of these 11 preparations have shown definite destruction of the cellulose. The distribution of these is indicated by the following table:

TABLE I.

Form	Number Tested	Number Showing Cellulose Digestion
Reticulotermes hesperis	8	2
Reticulotermes humilis	20	0
Porotermes froggatti	2	1
Kalotermes minor	4	3
Kalotermes hubbardi	20	0
Amitermes californicus	21	2
Termopsis angusticollis	6	1
Neotermes malatensis	4	2
	85	11

Cellulose digestion took place slowly in positive instances. The period of time necessary for disintegration to appear was of varying length and extended from 10 days to 3 months. The agent causing this digestion of the cellulose is transmissible since inoculums of disintegrating filter paper placed in other flasks of the medium induced the same change in them. Aerobic conditions are necessary inasmuch as anaerobic technique including the use of a paraffin-vasaline seal inhibits all action. The flora is made up of gram negative rods together with some micrococci. The dark field shows no spirals nor protozoa from these cultures.

All attempts to isolate in pure culture the organisms including this change were futile. The usual synthetic agar formula including precipitated granular cellulose within its structure yielded a variety of colonies aerobically although none appeared anaerobically in Burri tubes. No clear zone surrounded any of them and moreover mass inoculation into flasks of cellulose medium was of no effect. The addition of 0.02% sodium nitrate, of 0.1% glucose or of both to media solid or fluid did not improve the outcome. Rather, cellu-

lose destruction in the flasks proceeded better with neither of these compounds present.

Thus by methods probably somewhat unsuited to the material at hand but nevertheless adapted from procedures followed in soil bacteriology we have shown that there is a bacterial flora within the gut of certain individual termites which can destroy cellulose. In crude culture the action is slow. It is very possible that with media built up more closely in accordance with the environment of the intestine, this flora may appear more often and may show greater velocity in reactions produced. Our biochemical knowledge of this portion of the animal's anatomy, however, is as yet too fragmentary to proceed much farther at this moment.

4574

Duck Disease Studies I: Blood Analyses in Diseased Birds.

PAUL A. SHAW. (Introduced by C. D. Leake.)

From the Hooper Foundation for Medical Research of the University of California.

It has long been observed that ducks and other migratory birds which settle about inland marsh lands and shallow lakes reputed to contain "alkali waters" develop characteristic symptoms resulting frequently in heavy mortality. A systematic study of this disease has been proposed through the co-operation of the California Fish and Game Commission, the Hooper Foundation for Medical Research, and the Department of Pharmacology of the University of California Medical School. This report is the first of a series to be made in connection with this study.

This report deals with blood chemistry studies on diseased birds from the San Joaquin Valley district in comparison with normal healthy birds. Four blood constituents, non-protein-nitrogen, uric acid, blood sugar, and chlorides, have been studied on 13 normal and 15 diseased ducks of the pintail or sprig species *(Dafila acuta)*. Blood samples were taken directly from the heart and determinations made on protein-free filtrates according to the Folin and Wu system of analysis. Rectal temperatures were obtained preliminary to taking the sample.

The results indicate an average increase of 50% in non-protein-nitrogen, and an 80% increase of uric acid, in the diseased birds. In the chlorides, computed as sodium chloride, a barely significant

average increase was noted. The range of blood sugar values was too wide to warrant comparisons. The body temperature of sick specimens averaged 3.5° F. below normal. The results on normal birds are shown in Table I and those for diseased birds in Table II. In the diseased birds there appeared to be a relationship between non-protein-nitrogen, uric acid and body temperature, the figures obtained for these constituents increasing with increase of temperature. The 2 lowest figures for non-protein-nitrogen were obtained in the sickest birds, in one case the value lying below the minimum normal. The values were highest in the birds that were apparently nearing recovery, as evidenced by their ability to walk or fly.

TABLE I. *Normal Birds.*

Bird No.	Rectal Temp. °F.	Non-Protein-Nitrogen mg./100 cc.	Sugar mg./100 cc.	Uric acid mg./100 cc.	Chlorides % NaCl
1	110	43	120	9.2	0.41
2	111	40	138	6.6	0.48
3	110	39	157	9.3	0.44
4	109.5	39	151	6.7	0.47
5	109.1	39	148	7.6	0.44
6	108.5	40	136	4.5	0.48
7	110.4	38	177	6.4	0.47
8	108.8	38	172	4.5	0.50
9	107.5	35	148	4.4	0.47
10	108	37	179	6.4	0.47
11	111	40	208	8.0	0.46
12	109.3	37	220	6.1	0.48
13	109.5	40	154	7.0	0.44
Average	109.5	39	162	6.7	0.46

TABLE II. *Diseased Birds.*

Bird No.	Rectal Temp. °F.	Non-Protein-Nitrogen mg./100 cc.	Sugar mg./100 cc.	Uric acid mg./100 cc.	Chlorides % NaCl
1	104	48	300	—	0.46
2	105	36	163	9	0.45
3	102	28	200	10	0.53
4	106	52	160	11	0.56
5	108	68	138	10	0.47
6	107	60	145	12	0.47
7	108	56	126	10	0.47
8	107	80	153	15	0.44
9	104	58	200	10.5	0.51
10	108	68	143	11	0.47
11	106	52	162	12	0.52
12	106	55	150	8.5	0.49
13	108	59	204	12	0.55
14	105	144	137	34	0.60
15	107	53	153	10	0.49
Average	106	60	170	12	0.49

4575

Absorption of Agglutinins by "R" Variants of Bovine and Porcine Strains of Brucella Abortus.

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From the Division of Veterinary Science, University of California.

The serological identity of "normal" cultures of the porcine and bovine strains of *Brucella abortus* has been shown by Traum.[1]

The "R" variants of the porcine strain, although culturally and morphologically similar to those of the bovine strains previously reported,[2] appear to be more widely separated serologically from the bovine "R" cultures than are the parent cultures.

"R" variants of 7 strains of bovine source, 8 strains of porcine source and one strain from a spontaneously-infected laboratory guinea pig, were obtained by growing the cultures in broth plus 10% anti-*Brucella abortus* rabbit serum. These cultures included old stock cultures and recently-isolated cultures of both strains as well as cultures originally isolated in widely separated localities. Cells for absorption were obtained by growing the several cultures on 1% glucose-2% glycerine agar in sealed Blake bottles.

The absorption was accomplished by the addition to anti-serum of the same quantity of the various antigen suspensions which had been standardized to 1.5 mm. on the Gates opacimeter. In further absorption of the same serum, definite quantities of the standard suspensions were centrifuged until clear and the packed cells added to the serum.

Brucella abortus antisera from rabbits, cows and guinea pigs were used with results similar to those below. However, relatively low-titered anti "R" rabbit sera were most satisfactory for this differentiation. The absorbed sera were tested for agglutinins with our stock agglutinating fluid prepared from culture 80.

Table I shows the results of agglutination tests after the addition of several small absorbing doses of representative "R" antigens to anti 80 R serum.

The "R" variants of bovine source, with the exception of No. 80, removed the *Brucella abortus* agglutinins, while similar quantities of the "R" variant of porcine strains showed a much smaller absorbing capacity.

Of the 7 bovine strains tested, 5 showed relatively large absorbing

[1] Traum, J., *Annual Rep., Calif. Exp. Station*, 1921, 131.

[2] Henry, B. S., PROC. SOC. EXP. BIOL. AND MED., 1928, xxvi, 101.

TABLE I.

	Absorbed with				
	6 R	11 R	22 R	27 R	80 R
Bovine source	− − − − − − − −	− − − − − − − −	∓ − − − − − − −	− − − − − − − −	+ + + ± ∓ − − −
	1:100 1:160 1:200 1:320 1:400 1:640 1:800 1:1280				
Porcine source	+ − − − − − − −	+ + + + + + ∓ −	+ + + − − − − −	+ + + + + + ∓ −	+ + + + + + ± −
	402 R	407 R	408 R	411 R	Unabsorbed

capacity; one absorbed to a lesser degree than the other bovine strains, but more completely than did any of the porcine. No. 80 gave results similar to the porcine strains. This culture, although of bovine origin, has other characteristics of a porcine strain.

Of the strains of porcine source, 6 showed slight absorbing power, one (402) was only slightly less active than bovine strain 22, and one (409) gave a reaction similar to the bovine group.

The strain isolated from the spontaneously-infected guinea pig behaved like the majority of bovine origin.

The serologic difference shown here between the "R" variant suggests a division of the *Brucella abortus* strains into 2 groups.

4576

Maturation of Human Embryonic Ova.

OLIVE SWEZY AND HERBERT M. EVANS.

From the Department of Anatomy, University of California.

Sexual differentiation in the human embryo has taken place by the end of the seventh week of embryonic life. From this time until the third month simple growth and cellular multiplication occurs. With the third month the early maturation phases of the ova are found. These phases consist of the usual maturation phases present in the adult male germ cells, the formation of the leptonema, synizesis, pachynema, and diplonema, stages which do not occur in the adult female. Furthermore, in the development of the leptonema, prochromosomes like those of insects are formed and then resolved into the leptotene threads. Prochromosomes have not hitherto been seen in the mammalia. By five and one-half months the prochromosomes have nearly all disappeared and the type of maturation is the same as that in the adult male.

The appearance of prochromosomes seems to be a recapitulation stage such as is found in some other organs of the developing embryo. The maturation of embryonic ova may plausibly be due to the action of a maternal hormone incapable of affecting male embryonic germ cells.

The embryonic germ cells disappear before adult life is reached and the ova developed during adult life do not pass through the above maturation phases preliminary to the maturation divisions.

4577

Ovogenesis in the Mammalia.

OLIVE SWEZY AND HERBERT M. EVANS.

From the Department of Anatomy, University of California.

Ovogenesis occurs throughout adult life in the guinea pig, cat, dog, and man, as a rhythmical process, during which thousands of ova are produced *de novo,* followed by the degeneration of all but a few which mature. In the guinea pig, cat and dog this rhythm of ovogenesis coincides with the rhythm of the oestrus cycle, beginning at ovulation and reaching its peak at anoestrum, with wholesale degeneration occurring at late prooestrum. Lack of knowledge of a definite oestrus cycle in man weakens the correlation here, but the rhythm of ovogenesis is as striking in the number of ova produced and destroyed as in the other animals.

New sex cells are produced by proliferations from the germinal epithelium in the form of invaginations and ingrowths of epithelial cords. These become separated from the germinal epithelium, pass through the tunica albuginea and form a more or less continuous layer underneath the tunica. From one to many cells in each group may develop into ova, the remainder forming the follicle cells.

Contrary to the concept involved in the germ plasm theory, the mammalian ova (excepting those that mature and are fertilized) have a shorter life span than any other group of cells in the body outside of the reproductive tract.

4578

The Functional Difference Between the Pars Intermedia and Pars Nervosa of Hypophysis of Frog.

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From the University of California at Los Angeles.

Some years ago the writer demonstrated[1] that implantation of the combined *pars intermedia* and *pars nervosa* of adult frogs into tadpoles causes heavy pigmentation and transitory contraction of the body walls lasting several days. In a later paper[2] it was shown

[1] Allen, B. M., *Science,* 1920, N. S. Vol. lii, 274.

[2] Allen, B. M., *Anatom. Rec.,* 1925, xxxi, 302.

by transplantation of each part separated from the other, that the *pars intermedia* is alone involved in inducing pigmentation. The contraction of the body walls is conspicuous enough but difficult to measure. The object of the present paper is to present the results of such an evaluation. This was accomplished by measuring the area of the shadow cast by vertical illumination of each tadpole upon photographic print paper. Each print was projected upon paper by an opaque projection lantern and magnified 5 diameters. The area of each image was then measured with a planimeter, several such measurements of each tadpole used in the experiment being made at different times.

Transplants of *pars anterior, pars intermedia,* and *pars nervosa* were placed in a pocket under the skin over the right eye of *Rana aurora* tadpoles. This makes possible the location and later examination of the transplant in each case. The methods followed, though very laborious, do not give a quantitative measure of results but they do give a decision as to the effects of these implants upon body contour. It is felt that such painstaking studies upon a few specimens are more valuable than less careful studies of many. This method is considered preferable to volumetric methods because of the difficulty of separating water from the tadpoles without injury to them. Anaesthetization with ether in water may have slightly retarded growth during the period of the experiment, but the methods were impartially applied and it is felt that they serve as a fair index of the situation. The numbers of specimens of each type used were as follows: controls 6; *pars anterior* 9; *pars intermedia* 9; *pars nervosa* 9. Readings were made just before making the implant and at intervals of 2 days for 8 to 10 days later. Owing to some lack of uniformity in making later recordings, we shall take into account only the first 3 readings. In each case the rays were applied vertically in the dorso ventral plane of the tadpole. The results are shown in the following tabulation.

Effects of homoplastic transplantation of different portions of the hypophysis of adult *Rana aurora* frogs into tadpoles. Figures indicate average area of shadow.

Nature of transplant	Area on day of operation	Area after 2 days	Area after 4 days	% change after 2 days
Normal	.667 sq. c.	.664 sq. c.	.651 sq. c.	—0.45
Pars anterior	.702 sq. c.	.746 sq. c.	.766 sq. c.	+6.26
Pars intermedia	.784 sq. c.	.747 sq. c.	.748 sq. c.	—4.71
Pars nervosa	.765 sq. c.	.653 sq. c.	.675 sq. c.	—14.64

The slight contraction caused by *pars intermedia* transplantation and a slight temporary darkening of the surface of the tadpoles into which the *pars nervosa* is transplanted may be due to the presence of secretion which has been interchanged between these two intimately associated structures by diffusion. In the case of the *pars intermedia* transplants pigmentation becomes progressively deeper until at the end of 3 or 4 weeks it is extremely intense. Transplantation of the *pars nervosa* never gives more than a slight temporary deepening of color that disappears completely in 2 or 3 days.

We believe that the above data strongly point to a specific influence exerted by the *pars nervosa* upon the distension of the body wall.

4579

Do Foreign Proteins Multiply in the Animal Body?

W. H. MANWARING, J. L. AZEVEDO AND T. H. BOONE.

From the Laboratory of Bacteriology and Experimental Pathology, Stanford University, California.

If 2 cc. horse serum per kilo of body weight are injected subcutaneously, in minute divided doses, into a normal dog, and, if the absorption of the injected horse proteins into the canine circulation is followed by quantitative tests with rabbit precipitin, data are obtained that suggest a 100% absorption of the injected horse proteins into the blood stream by the end of 4 to 7 days, rising to a 200%, or even a 400% absorption by the 14th to 21st day. A 200% horse protein assay of the blood alone might well mean a 1000% or even 2000% alien protein content of the body as a whole.

This does not necessarily mean that the injected horse proteins multiply as a living virus in canine tissues, a biochemical metaphor thus far suggested solely for the bacteriophage. Among the conceivable alternate explanations are: (a) apparent multiplications due to hydrolysis or colloidal dispersion of the injected horse proteins, (b) the formation of pseudo-horse proteins as a result of denaturization of the body proteins of the injected dog, (c) the synthesis or liberation of antibodies of approximate horse protein specificity, and (d) toxic increases in some hypothetical non-specific precipitin reaction.

This paper represents selected data from over 50 experimental and control dogs.

4580

The Alien Globulin-Albumin Ratio in Artificial Serum Mixtures.

J. L. AZEVEDO. (Introduced by W. H. Manwaring.)

From the Laboratory of Bacteriology and Experimental Pathology, Stanford University, California.

If 5 cc. 7.5% horse serum-globulin are added to 95 cc. normal dog serum, the mixture allowed to stand in the ice chest over night, and the proteins of the mixture then separated by half-saturation, followed by full-saturation with ammonium sulphate, titrations by means of specific rabbit precipitin indicate a quantitative recovery of the horse protein in the globulin fraction of the serum mixture.

If horse serum-albumin is similarly added to normal dog serum, 33⅓% of the horse protein is recovered in the globulin fraction, and 66⅔% is the albumin fraction. A similar apparent 33⅓% conversion of horse albumin into horse globulin takes place in heat-inactivated normal dog serum.

The above tests are preliminary to a study of the mechanism of the intravenous denaturization of foreign proteins.

4581

Is a Quantitative Precipitin Titration Possible?

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From the Laboratory of Bacteriology and Experimental Pathology, Stanford University, California.

In order to estimate the specific protein content of an unknown solution, 2 methods of precipitin titration are in use. Successive dilutions of the unknown may be mixed with a constant amount of specific antiserum, and the maximum dilution giving a demonstrable precipitin reaction may be determined. This dilution is compared with the maximum dilution of a known or standard protein solution giving the same end-reaction. For example, if the unknown gives an end-reaction in the dilution 1:1000, while the corresponding reading with the standard solution is 1:10,000, the conclusion is drawn that the unknown contains 10% of the specific protein of the standard, interfering factors, of course, being experimentally ruled out.

The method favored by many botanists and zoologists, however, is to estimate the amount of precipitate in some arbitrary dilution, preferably by means of a hematocrite,[1] the assumption being that equal precipitates indicate equal amounts of specific protein.

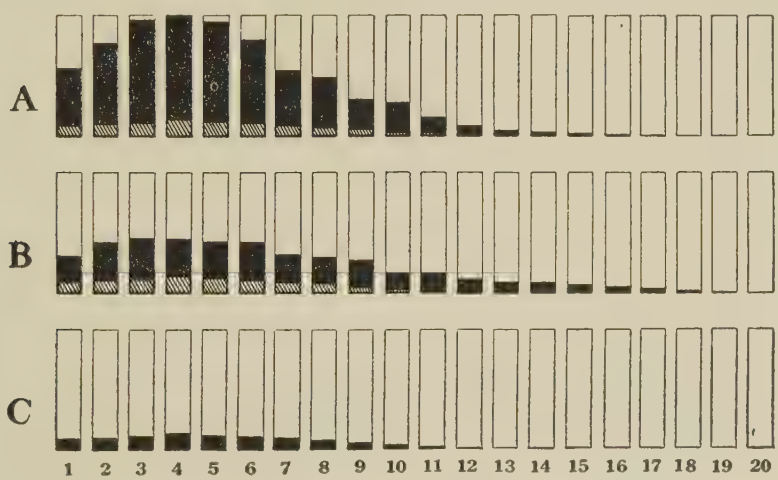
Applying these methods to a study of the parenteral history of alien proteins we have obtained contradictory and paradoxical results, the details of which have not yet been published.[2] As an illustration, the following data are cited.

If 2 cc. horse serum per kilo of body weight are injected intravenously into a normal dog, and if blood samples are withdrawn from this dog: (i) immediately before the injection, (ii) 15 minutes after the injection, and (iii) at the end of about 14 days, parallel titrations of the sera of the resulting blood samples by means of an ice-chest ripened (14 to 30 days) specific rabbit precipitin give the readings recorded in Fig. 1.

FIG. 1.

Attempted Titration of 14-day Parenteral Alien Proteins.

0.5 cc. successive dilutions (1:2) of the serum unknown, plus 0.5 cc. 20% rabbit antiserum; incubator 2 hours, ice chest over night. Each tube is now shaken to a uniform turbidity. Three of the maximum turbidities are mixed to form the 100% turbidity standard, the turbidities being read as percentages of this standard.



A—Serum from 15-minute canine blood sample.
B—Serum from 14-day canine blood sample.
C—Control, normal dog serum.

Cross hatched portions of A and B represent the "non-specific precipitate" (see C), which presumably must be subtracted from the total precipitate, to give specific protein reaction (black). Well ripened rabbit precipitin usually gives much less "non-specific precipitate" than indicated in C.

[1] Boyden, A., and Baier, J. G., Jr., *J. Immunol.*, 1929, xvii, 29.

[2] Manwaring, W. H., *Science*, 1929, lxx, 2.

Comparison of the titers or end-reactions suggests that Sample B has at least 4 times the horse protein content of Sample A. Comparison of turbidities in the 9th to 12th dilutions suggests that A and B are of approximately equal horse protein content. Lower dilutions indicate that A has at least three times the horse protein content of B.

Our current quantitative precipitin technic apparently requires further study before it is applicable to complex protein mixtures.

New York Meeting.

New York Academy of Medicine, October 16, 1929.

4582

Toxic Products in Infected Pork.

G. H. ROBINSON AND F. A. TAYLOR. (Introduced by Samuel R. Haythorn.)

From the Wm. H. Singer Memorial Research Laboratory of the Allegheny General Hospital, Pittsburgh, Pa.

Recent investigations have disproved the theory that living pathogenic organisms must be present in food in order to cause that train of symptoms usually associated with food poisoning. The work of Ecker and his associates,[1] Branham[2] and Geiger[3] clearly indicates that organisms of the paratyphoid group are capable of producing a heat resistant toxin in culture media. Dack, Jordan and Wood[4] have demonstrated that the killed bodies of paratyphoid bacilli are not toxic when ingested. The report of Pryer[5] shows that meat from which no organism of known pathogenicity could be isolated was toxic for human beings. Organisms other than those of the paratyphoid group such as *B. proteus* have been suspected of causing food poisoning.

We have also found *B. proteus* predominating in some raw sausages which after apparent sufficient cooking caused intestinal disturbance. It seemed of interest to us to investigate the nature of a toxin, if any, produced on meat and fish by *B. proteus, B. paratyphosus B.* and *Staphylococcus albus.* At first fresh meat was inoculated with our test organisms but was found to be so grossly contaminated that no conclusions were possible. Our results in these

[1] Ecker, E. E., *J. Infect. Dis.*, 1917, xxi, 541. Ecker, E. E., and Megrail, E., *Ibid.*, 1925, xxxvii, 546.

[2] Branham, S. E., *Ibid.*, 1925, xxxvii, 308.

[3] Geiger, J. C., and Meyer, K. F., PROC. SOC. EXP. BIOL. AND MED., 1928, xxvi, 91.

[4] Dack, G. M., Jordan, E. O., and Wood, W. L., *Ibid.*, 1929, xxvi, 307.

[5] Pryer, R. W., *Am. J. Pub. Health*, 1919, xix, 208.

experiments were obtained from meat which was inoculated after autoclaving at 15 pounds for 15 minutes.

After inoculation the meat was kept at room temperature for periods of from 3 to 19 days, after which it was ground in a mortar and extracted with either alcohol or water. Water extracts were heated to 70° for 30 minutes. Alcoholic or ether extracts were evaporated to dryness *in vacuo* and resuspended in salt solution. Toxicity was determined by the intraperitoneal injection into young rats or white mice.

The toxic substance is readily produced in pork and fish by *B. paratyphosus B.* or *B. proteus* but not by *Staphylococcus albus.* It was not recovered from uninoculated controls. As has been found by others, it is heat resistant, withstanding 100° C. for 30 minutes. It is soluble in water, alcohol and ether. More refined chemical methods may demonstrate two toxic substances, one of which causes diarrhea and the other prostration and death. It was quite noticeable that water extracts produced death while alcohol and ether soluble fractions gave rise to a transient diarrhea.

It is apparently not a protein as it is soluble in absolute alcohol and is not precipitated by sodium tungstate. It will pass through a Mandler filter. In attempts to produce an antitoxin in rabbits treated with a toxic water extract no protective action could be demonstrated by the serum of these animals.

A toxin is produced in cooked pork and fish by *B. paratyphosus B.* and *B. proteus* but not by *Staphylococcus albus.* This toxin is heat resistant and is probably not a protein. Attempts to produce an antitoxin were unsuccessful.

4583

Ephedrine Hydrochloride on the Excised Ureter of the Dog.

GEORGE B. ROTH.

From the Department of Pharmacology, George Washington University Medical School, Washington, D. C.

Apparently the only observation thus far made of the effects of ephedrine on the ureter is that reported by J. Hofbauer.[1]

Hofbauer obtained a stimulating effect from ephedrine on both longitudinal and ring preparations of the excised ureter of the pig.

[1] Hofbauer, J., *J. Urology*, 1928, xx, 413.

The degree of stimulation produced was less than that produced by epinephrine. The only concentration of ephedrine which he seems to have employed was a rather high dilution of the drug, namely, "0.3% ephedrine in 50 cc. of oxygenated Locke's solution."

The present report deals with the effect of both dilute and concentrated solutions of ephedrine hydrochloride on the upper two-thirds of the excised ureter of the dog, when the segment was suspended in Locke-Ringers' solution, the pH of which was 8.2.

The preparation of ephedrine hydrochloride was obtained from Dr. Bernard E. Read of the Department of Pharmacology, Peking-Union Medical College. It had the following properties: Melting point, 215° to 216° C.; optical rotation, —32.2° to —32.5°.

The usual effect on the dog's ureter may be seen from the tracing of one experiment with one dose.

TRACING NO. 1.
Effect of 5 mgm. of ephedrine hydrochloride on the surviving ureter of the dog. Time in minutes.

The range of dosage extended from 1 to 90 mg. of ephedrine hydrochloride in 100 cc. of Locke-Ringer's solution, the former dilution closely approaching that used by Hofbauer. The concentrated solutions were employed in order to determine whether the dominant effect on the ureter was stimulation or depression.

Conclusions: 1. Ephedrine hydrochloride is mainly stimulant to the excised ureter of the dog. 2. The qualitative effect of ephedrine hydrochloride on the dog's ureter closely resembles that produced by epinephrine. 3. Ephedrine hydrochloride and epinephrine differ widely quantitatively when measured by the reaction of the surviving ureter to these agents, the latter being in some experiments

about 200 times as stimulant as ephedrine. 4. The depression of the dog's ureter which appears after the use of the more concentrated solutions of ephedrine hydrochloride, invariably follows a rather extended period of stimulation, a period in which tonus, rate and amplitude of contraction, either singly or collectively, may be increased.

4584

Cell Proliferation Response to Sulphydryl in Man.*

STANLEY P. REIMANN AND FREDERICK S. HAMMETT.

From the Research Institute of the Lankenau Hospital, Philadelphia.

Hammett[1] has shown in studies from this Institute that the radicle –SH is the essential stimulus to cell division in normal growth by cell number in certain plants and paramecia. Extension of this work to a mammal (rat) has shown that solutions containing this radicle, stimulate cell division in the healing of wounds.[2] This report embodies the work on man.

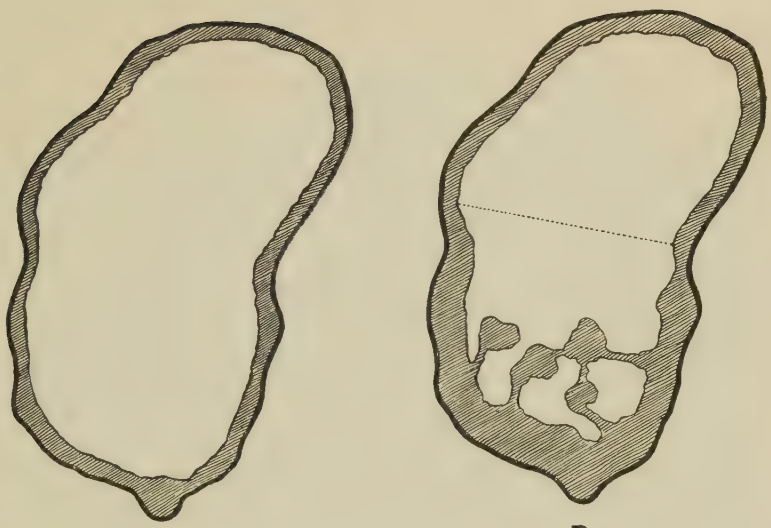
A male, 76 years old, fracture upper third of the thigh, had an ordinary varicose leg ulcer on the same leg, one half way between the knee and ankle. This ulcer was divided in half by a dam of adhesive plaster and one part treated with a solution of thio-glucose,[2] while the other half was treated by painting with 5% mercurochrome solution. The ulcer had existed for over 10 years, had never healed despite numerous and various treatments and with short periods of improvement, continued to progress. At the end of 24 hours, the ulcer was sharply divided by a growth in the sulphydryl treated part consisting not only of granulations but also of thin, translucent, ground-glass-like epithelium growing in from the edges and in islands in the ulcer. The original depth of 1/8 to 1/16 inch had filled to the surface with granulations at points devoid of epithelium to almost the point of exuberance. The other half of the ulcer showed no progress.

A woman, 74 years old, with a fracture of the hip, developed a

* Since these experiments were done, increased cell proliferation in root tips has been obtained with the same compound, *viz.*, thio-glucose.

[1] Hammett, F. S., *Protoplasma*, 1929, vii, 297; *Trans. Am. Philosoph. Soc.*, 1929, lxviii, 151.

[2] Hammett, F. S., and Reimann, S. P., *J. Exp. Med.*, 1929, l, 445.



A. Exact shape of leg ulcer obtained by tracing. Reduced approximately one-half. Shaded part is the newer epithelium blending into surrounding skin.

B. Twenty-four hours later. Lower part treated with thio-glucose solution. Shaded areas epithelium. No idea of depth is given in illustration but in the lower part the unshaded areas are healthy appearing granulations flush with or slightly above the general surface level. Exact shape, reduced approximately one-half.

typical decubitus ulcer over the sacrum. This was treated for 3 weeks by various standard methods with hardly any improvement. The edges were undermined to a depth of one-quarter inch. Application of wet dressings of thio-glucose solution stimulated healing to the extent that over one-quarter of the circumference had grown fast to the underlying granulations in 24 hours and a growth of approximately 1/16 of an inch of epithelium occurred around the rims. Because of the presence of abundant superficial pus, treatment was discontinued temporarily.

A man, 62 years old, suffered from "trophic" ulcer over the head of the fifth metatarsal, right foot. The wound looked like an old, chronic, calloused gastric ulcer about ¾ inch in diameter and ¾ inch deep. Various methods of treatment over a period of 6 weeks hardly influenced its status. Wet dressings of thio-glucose solution for 48 hours resulted in a wound ½ inch in diameter and about ¼ inch deep, growth occurring not only of granulations but also of epithelium. There was abundant surface pus.

A man, 66 years old, had 2 varicose ulcers, one on each side of the left ankle. Healing had begun on the internal one but was markedly stimulated by application of thio-glucose for 48 hours, whereupon

the surface pus again appeared in abundance and the treatment was discontinued.

The main interest at this Institute in these results, lies not so much in their obvious practical application as in the demonstration, the essential validity of the sulphydryl theory of growth by increase in cell number as proved in plants, uni-cellular animals and the rat.[1]

As far as the clinical use of this radicle is concerned in the stimulation of healing, many practical details remain to be investigated. Thio-glucose apparently stimulates bacteria as well as cells and the presence of this surface infection of itself inhibits healing.[3]

Perhaps thio-phenol or thio-cresol or some similar compounds will answer this objection. There are also investigations necessary as to the advantages of drip methods, applications on gauze, etc. In root tips, Hammett[4] has found that cells stimulated to rapid division by sulphydryl compounds are quite small in size. This is probably because the nuclei are stimulated to divide before the cytoplasm has a chance to absorb ordinary nutrition and reach its normal size. This may perhaps be "poor healing" because of the smallness of the newly divided cells.

A few preliminary experiments on other wounds have shown that it is advantageous to use sulphydryl solution for about 24 hours with intervals of 2 or 3 days between, when wet dressings of boric acid solution or other similar substances can be applied. Under such circumstances several wounds have healed in spurts, so to speak.

Summary: Thio-glucose in solutions of 1 to 10,000 applied as wet dressings, stimulates the growth of both granulation tissue and epithelium in wounds. Many details await investigation before this can be put to its most advantageous practical use. The evidence here presented is further proof of the sulphydryl theory of growth by increase in cell number.

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[3] Carrel, A., and Hartmann, A., *J. Exp. Med.*, 1916, xxiv, 429.

[4] Hammett, F. S., *Protoplasma*, 1929, in press.

4585

Basal Gaseous Metabolism of Giant Rats.

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From the Memorial Foundation for Neuro-Endocrine Research and the Laboratory for Surgical Research, Harvard Medical School, Boston.

Gigantism can be produced in rats by the administration of extracts of the anterior lobe of the pituitary.[1] We have determined the basal gaseous metabolism in 4 such giant rats and have compared their metabolic rates with those of 4 control females of the same age and the same Wistar strain.

The rats were approximately 11 months old at the beginning of the metabolism determinations. Since the age of 7 months, each of the experimental animals had received daily parenteral injections of 1 cc. of a neutralized alkaline extract of fresh beef anterior lobe containing the growth promoting principle.[2] During the period of the metabolism determinations the weight range of the injected rats, before being starved, was from 350 to 530 gm. The largest of the control rats were selected in order to obviate as much as possible the effect of size *per se* on metabolism. Their weights ranged from 200 to 270 gm. The increased size and weight of the injected animals is apparently due to the greater bone, muscle and skin development and to the considerable increase in size of most of the viscera, especially the liver. Body fat does not appear to be increased. Despite the obvious general overgrowth, the body lengths of the injected rats averaged less than 10% more than those of the controls.

Metabolic rate determinations were made by the oxygen consumption closed circuit method at weekly intervals. Each determination represents the average rate, in several tests, over a 3 to 4 hour period, and 18 to 22 hours after the last ingestion of food. All tests included were considered satisfactory from a technical standpoint. Body surface was calculated from the formula[3]

$$S = 10.76\, W^{0.61}\left(\frac{0.31}{\frac{W^{1/3}}{L}}\right)$$

Rat 62 was sacrificed for direct measurement of the skin area, which was found to be only 4% greater than that predicted by the for-

[1] Evans and Long, *Anat. Rec.*, 1921, xxi, 62.

[2] Putnam, Teel and Benedict, *Am. J. Physiol.*, 1928, lxxxiv, 157.

[3] Lee and Clark, *Am. J. Physiol.*, 1929, lxxxix, 24.

mula. Respiratory quotients were found to be nearly 0.72 in both the injected and control starved rats.

TABLE I.
Basal Metabolic Rates of Giant Rats.

Rat No.	With Extract			Without Extract		
	Weight range (gm.)	No. of determinations	Cals., day, sq. m.	Weight range (gm.)	No. of determinations	Cals., day, sq. m.
14	479-512	4	703			
62	424-462	5	705	425-370	4	801
94	336-350	3	696			
12	400-403	4	683	354-323	3	787

The average heat production of the 4 injected rats, in 16 determinations, over a period of 5 weeks, was 697 calories per day per square meter, with a standard deviation of ±21 calories. The 4 control rats in 9 determinations gave a metabolic rate of 805 calories with a standard deviation of ±50 calories. This rate agrees well with the average of a large number of determinations on other normal rats under the same conditions. Injections in rats 62 and 12 were stopped and after a rest period of 3 weeks the metabolic rates were found to be decidedly higher, in fact within the normal range.

These results in themselves do not prove that the anterior lobe growth hormone is anabolic or metabolism sparing in its action. There may not have been an increase in metabolically active body tissues in the giants to the extent indicated by the increased weight. There is apparently some hydration of the tissues, as indicated by the fact that these rats when starved for 22 hours lost much more weight proportionally than did their controls. There is also a rapid loss in weight during the first few days after cessation of the injections. Further work is in progress to determine more exactly the calorigenic effects of these principles.

4586

Normal Tetracosanic Acid.

F. A. TAYLOR. (Introduced by Samuel R. Haythorn.)

From the William H. Singer Memorial Research Laboratory of the Allegheny General Hospital, Pittsburgh, Pa.

In connection with studies of the structures of the fatty acids of cerebrosides[1] and their relationship to the lignoceric acid of peanut oil, it became imperative to examine the latter source more carefully than had heretofore been done. The results so far obtained are of sufficient importance to warrant the publication of this note.

In the usual preparation of lignoceric acid from peanut oil, the saturated acids are fractionated from various solvents until the least soluble fraction melts at 80-81°. Further crystallization produces no further change.[2]

In the present experiments a systematic fractional distillation[1] was carried out which might be expected to produce a quite different distribution of the individual acids than would crystallization. Small fractions of highest and lowest volatility, respectively, were removed at each distillation, the large middle fraction serving for the next following distillation. The free acids were then grouped according to molecular weight and melting point and crystallized from large volumes of ether, first at room temperature and finally at 2-5° C.

From the higher fractions an acid was obtained which agrees closely in properties with the normal tetracosanic acid. It melts at 84-85° and its molecular weight by titration is 372. Levene and Taylor[3] found for the synthetic normal acid a melting point of 85-86°. Several crystallizations from ether brought about no further change in the acid. It has been obtained from 2 samples of peanut oil. The agreement in melting point is sufficiently close if one considers the difficulty of separating individual fatty acids in a perfectly pure state from mixtures.

It should be noted that the normal acid was not obtained from lignoceric acid but from the mixture of acids which give rise to lignoceric on repeated fractional crystallization. The relationship to lignoceric acid can only be determined by the preparation of pure lignoceric and its fractional distillation. Experiments on this phase of the problem are in progress.

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[1] Taylor, F. A., and Levene, P. A., *J. Biol. Chem.*, in press.

[2] Levene, P. A., Taylor, F. A., and Haller, H. L., *J. Biol. Chem.*, 1924, lxi, 157.

[3] Levene, P. A., and Taylor, F. A., *J. Biol. Chem.*, 1924, lix, 905.

4587

Basal Metabolic Rate in Advanced Age.

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The number of measurements which have been recorded of the basal metabolic rate in persons over 70 is small. Magnus-Levy and Falk measured 5 men of the ages of 64 to 78 and 7 women of the ages of 71 to 86. Aub and DuBois examined 6 old men with obvious stigmata of senility. With all these cases there was a definite lowering of the basal metabolic rate below the predicted. In one case in the Aub and DuBois series the rate was —30.

In contrast to these stand the measurements by Dr. Stoner made for Professor F. G. Benedict on Professor Keen, aged 89. With the 3 standard methods of prediction, Professor Keen's basal metabolism was above the predicted. With the Harris-Benedict method the basal metabolic rate was +26.2. Benedict is inclined to believe that this represents more accurately the physical condition of Dr. Keen than do the other two.

I have examined the basal metabolic rate of 4 persons whose ages range from 72 to 89. The subjects were 3 men of great mental and physical vigor, one of these was a person standing 6 ft. 1 inch in height. The woman examined is unusually small, who has never had any illness during 35 years service in my family, is physically most active and mentally highly acute.

TABLE I.

Subject	Age	Weight	Height	Harris-Benedict	Aub & DuBois	Dreyer
		kgm.	cm.	%	%	%
W. W. K.	89	60.3	154	+26.2	+2.9	+1.1
J. C-B.	89	58.8	172	+15	—5.7	—2.7
A. C. H.	74	71.3	178	+ 5	—5.5	+1
E. W. H.	72	59.4	186	+ 5	—9.2	+2.5
S. K.	79	41.6	149	+ 9	+3.2	+6.0

Note. The Aub and DuBois values for W. W. K. and J. C-B. are based on 34.5 cals. per M^2 per hr. and for S. K. on 35.5 less 7% = 33.5 cals. per M^2 per hr.

In the case of J. C-B. 6 measurements were made, 2 measurements per day being made on 3 different days. In the case of S. K. 2 measurements were made on each of 2 successive days. In the other cases 2 measurements were made on a single day. The meas-

urements were made with the Benedict Student Apparatus in the strictly post absorptive condition. In no case was a heavy meal taken the previous night. The subjects were examined in their own homes after an undisturbed night's rest. The position adopted was the recumbent one. For the purposes of comparison I have entered the results on Professor W. W. Keen. In none of these cases is there any obvious evidence of a decreased metabolic rate as the result of advanced age. The single prediction which falls much below the centre point is that of E. W. H., whose rate is —9.2 by Aub and DuBois' prediction. This may be accounted for by his unusual configuration owing to his great height and slight weight.

In Aub and DuBois' discussion, the lowering of the metabolic rate is assumed on the basis of 39.7 calories per M^2 per hour, a value which is the average for subjects between the ages of 20 and 50. The average lowering was 12%. If one were to calculate Professor Keen's heat exchange on this basis he would give —10.6 and J. C-B. —17.6%.

With these subjects the exchange averages about 2 cals. per M^2 per hour lower than the men of Aub and DuBois' series. This may be due partly to the method of measurement and partly to the very complete absence of movement which was obtained with my exceptionally intelligent and co-operating group. The very small and active female subject gives a value close to that obtained with Professor Keen.

The results obtained do not afford any ground for assuming that the slope of the curve of Aub and DuBois for subjects between 20 and 70 years of age changes after the latter age is reached.

4588

Spectrographic Examination of Pellagrins' Sera.

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From the Departments of Tropical Medicine, Internal Medicine and Physiology, Tulane University School of Medicine.

That exposure to direct sunlight produces or intensifies the erythematous eruptions on the exposed parts of the bodies of pellagrins is an opinion of many observers; the underlying cause of this apparent photo-sensitization is, however, entirely obscure. The auto-experiment of Meyer-Betz[1] with hematoporphyrin and the skin manifesta-

[1] Meyer-Betz, F., *Deutsches Archiv. f. Klin. Med.*, 1913, cxii, 476.

tions of hydroa-aestivalis, buckwheat disease, and other forms of apparent sensitization among the lower animals[2] at least justify the suspicion that some toxic substance is circulating in the blood stream.

With the object of determining whether or not the spectrum of pellagrous differed materially from that of non-pellagrous serum, a series of 13 sera was examined with a Hilger quartz spectrograph.

Each case had been admitted to the New Orleans Charity Hospital and all were in the acute stage with characteristic eruption, usually with marked oral and gastro-intestinal symptoms. The serum was obtained in the following manner: After a 12 hour fast blood was drawn from the median basilic vein, using a dry needle and glass syringe. Coagulation was allowed to take place in a paraffin coated tube. The serum was pipetted off and centrifuged at 3000 R.P.M. for 10 minutes and again pipetted off.

Our principal endeavor was to prove the presence or absence of hematoporphyrin in the serum, and our second to detect if possible any deviation of the spectrum of pellagrous from that of normal serum. The instruments used were a large E 4 Hilger quartz spectrograph and a quartz cell measuring ¾″ by ¾″ by ⅛″ internal diameter. An arc between 2 adjustable rods of soft Norwegian iron served as the source of radiation. This arc (Fig. 1) affords an efficient source not only for the visible, but for the ultra violet part of the spectrum as well.

In no instance were we able to detect sufficient difference between the spectra of the normal and the pellagrous sera to warrant the con-

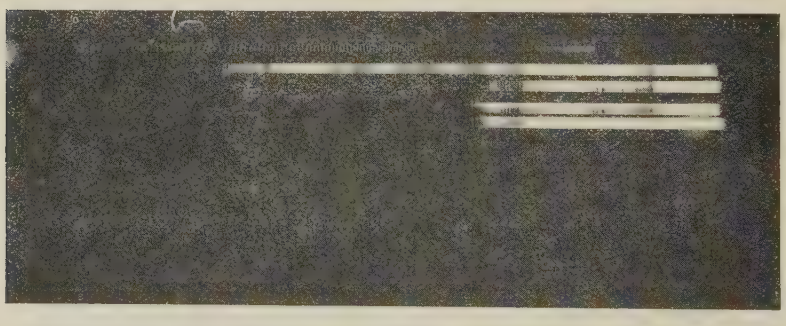

FIG. 1.

Spectrograms of normal and pellagrous serum. The first spectrogram is of the iron arc (6 A, 35V), exposure of 20 seconds. The second spectrogram is of a sample of normal serum, 3 minutes exposure. The next two spectrograms are of samples of pellagrous serum taken from two different patients, 3 minutes exposure.

[2] Hausmann, W., *Grundzüge der Lichtbiologie und Lichtpathologie.*

clusion that they were not spectroscopically identical. Furthermore, comparison between normal serum containing traces of hematoporphyrin and pellagrous serum left no room for doubt that this substance, at least in any detectable quantity, is not present in the circulating blood of pellagra victims.

Three and one half liters of urine from an acute case were concentrated *in vacuo*, extracted with alcohol, the residue from the alcohol evaporation taken up with acid, and the examination carried out in the usual manner. The spectrogram showed no evidence of the presence of hematoporphyrin.

4589

A Crystalline Substance of the Hypophysis Which Promotes Follicular Growth.

PEARL E. CLAUS. (Introduced by M. F. Guyer.)

From the Department of Zoology, University of Wisconsin.

While working with an acid alcohol extract of the anterior lobe of the pituitary body, according to the earlier method of Hisaw, Fevold, and Meyer,[1] it was found possible to produce 2 very different ovarian reactions depending upon the dosage used. When a *small* amount of the extract was injected into adult rats, follicular growth increased and the rats remained in a condition of oestrum much longer than normally. On the other hand, if a *larger* amount of the same extract was injected the follicles in the ovary became atretic with the formation of corpora lutea without ovulation, the animal remaining in a state of dioestrum.

Attempts were then made to separate the extract into fractions which would give one of the above mentioned physiological effects without the other. This was done by following the unpublished technique used by Hisaw in making an extract of corpus luteum hormone. An acid alcohol extraction of desiccated anterior lobe was, during the process of purification, separated into one fraction which was soluble in absolute alcohol and one which was not. From the latter a crystalline product was secured which induces precocious sexual maturity. Such crystals when injected in aqueous solution into 18 day old mice, in amount equivalent to ½ gm. of fresh material per day, invariably caused the vagina to open in from 2 to 3

[1] Hisaw, F. L., Fevold, H. L., and Meyer, R. K., *Phy. Zool.*, 1929, in press.

days. Normally the vagina remains closed until about the thirty-fifth day (30th to 49th day). The ovaries of the treated mice showed numerous and large mature follicles and in those animals which were not killed ovulation always occurred.

However, when an equal, daily amount of the fraction soluble in absolute alcohol was injected into similarly immature mice, the vagina did not open until the animals were from 31 to 37 days old, which is about the normal age of sexual maturity. The large follicles of such individuals became filled with lutein tissue, the ova within disintegrated, and no ovulation took place. A cross section of the ovary of such an animal looks much like that described by Aschheim and Zondek[2] in mice injected with urine from pregnant women, while the cross section of an ovary of a mouse injected with the crystalline fraction resembles that found by Smith and Engle,[3] after daily transplant of the anterior lobe. It may be that the secret of the apparent divergence in the results of these respective investigators lies in the existence of 2 different substances as indicated by the fractions just described.

By administering daily transplants of the anterior lobe of the pituitary, Smith and Engle, for instance, induced precocious maturity in female mice and rats with increased follicular growth and subsequent ovulation. Aschheim and Zondek, on the contrary, working with urine of pregnant women, which is supposed to contain anterior lobe hormone, found quite a different picture in the ovaries of injected mice. The large, abundant follicles became hemorrhagic, forming what they termed "blutpunkte". Luteinization of the follicle and degeneration of the ovum occurred producing corpora lutea without ovulation. This, Aschheim and Zondek attributed to the anterior pituitary hormone in the urine.

Evans and Simpson[4] recently recognized 2 hormones in the anterior lobe: one a maturity producing substance, which was found in acid aqueous extract of the anterior hypophysis, in the placenta, and in urine from pregnant women, the other obtained in alkaline aqueous extract, a growth producing hormone which at the same time caused luteinization of the follicles and inhibition of ovulation. The former seems to agree more closely with the crystalline fraction previously mentioned. Other experiments are being carried on at the present time to test further physiological effects of the substances described in this paper.

[2] Aschheim, S., and Zondek, B., *Klin. Wchnschr.*, 1928, vii, 1404.

[3] Smith, P. E., and Engle, E. T., *Am. J. Anat.*, 1928, xl, 159.

[4] Evans, H. M., and Simpson, M. E., *J. Am. Med. Assn.*, 1928, xc, Nr. 18.

4590

Insulin and CO_2 Combining Power of Blood Plasma in Normal Dogs.

ROBERT M. HILL AND WILLIAM B. DRAPER.
(Introduced by Robert C. Lewis.)

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In connection with other work in this laboratory we determined the CO_2 combining power of the blood plasma in normal dogs at intervals after the administration of insulin subcutaneously. We were surprised to find that after a slight rise in the CO_2 combining power a much greater fall always occurred.

The dogs used were fasted from the previous day. The experiments were started, usually, about 10:00 A. M. From the table it may be seen that the blood samples were drawn at irregular intervals. After several experiments these sampling times were chosen because of the short duration in the rise and the subsequent prolonged fall in the CO_2 combining power.

By reference to the table on page 32 it is seen that the slight rise in the CO_2 combining power, occurring from 40 to 50 minutes after the injection of insulin, is a constant phenomenon and that it occurs at nearly the same time that the blood sugar begins to fall. This might be due to the burning of the residues of fat metabolism thus freeing some base in the blood. On *a priori* grounds we would assume that the fall in the CO_2 combining power of the plasma is a phenomenon of a different nature.

It is becoming common practice to treat acidosis, whether due to ketogenesis or not, by means of insulin. Our results indicate that insulin may intensify a non-ketogenic acidosis.

Further work on this problem is in progress in our laboratories.

TABLE I.

Changes in glucose of blood and CO_2 combining power of blood plasma after subcutaneous injection of insulin in normal dogs.

											Wt. kg.	Insulin units
Dog 101	Minutes after Injection	0	25	50	120							
	Glucose, mgm. %	80	79	63	50							
	CO_2, vol %	46	48	56	39						17.5	35
Dog 102	Minutes after Injection	0	40	50	60	120	210	265				
	Glucose, mgm. %	86	91	74	42	41	40	36				
	CO_2, vol %	42	47	47	43	42	37	32			15.0	30
Dog 103	Minutes after Injection	0	30	46	60	120	180					
	Glucose, mgm. %	88	83	68	55	50	50					
	CO_2, vol %	43	46	47	37	33	32				15.0	30
Dog 104	Minutes after Injection	0	30	45	60	120	180	240*	270	305		
	Glucose, mgm. %	70	47	41	38	40	34	32	90	167		
	CO_2, vol %	50	53	53	39	31	37	37	39	46	22.0	62

*At 246 minutes, 25 gm. of glucose were given by stomach tube.

4591

Pituitary Extract and the CO_2 Combining Power of the Blood Plasma.

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(Introduced by Robert C. Lewis.)

From the Departments of Physiology and Pharmacology and Biochemistry, University of Colorado School of Medicine, Denver, Colorado.

It has long been known that the injection of pituitary extract results in a marked rise in the level of the blood sugar. For this and other reasons the posterior lobe of the pituitary gland has been credited with some obscure rôle in carbohydrate metabolism. Such a physiological action on the part of extracts of the gland would appear to suggest a related action on the CO_2 combining power of the blood plasma and possibly on other properties and constituents of the blood. This problem is now under investigation in our laboratories.

Three typical experiments are given in the accompanying table. From Experiment No. 4, it will be seen that the intravenous injection of commercial pituitary extract is followed immediately by a marked fall in the CO_2 combining power of the blood plasma. In this experiment the extremely low level of 21.5 volumes per cent was reached in 10 minutes. Sixty minutes following the injection, the CO_2 combining power had risen to 40.9 volumes per cent. In view of the great rapidity of this fall it does not seem likely that ketogenesis is a factor.

The distribution of this acidosis-producing hormone between "Pitocin"* and "Vasopressin" has been determined. Reference to the table, Experiment 13, shows that the power to produce a fall in CO_2 combining power and a rise in the blood sugar is present in the preparation "Pitocin" which contains less than 1% of the normal pressor activity. This action, although similar, is not so marked as that possessed by "Vasopressin", a preparation which is nearly free from oxytocic activity. The hormone in pituitary extracts which produces this acidosis and rise in blood sugar is, therefore, in all probability, chemically separate from both the pressor and oxytocic hormones.

Our experiments have not at this time progressed sufficiently far to indicate whether this hormone is identical with or separate from the diuretic-antidiuretic hormone.

* Furnished by the courtesy of Parke, Davis & Co.

TABLE I.

Changes in glucose of the blood and CO_2 combining power of the blood plasma after intravenous injection of pituitary extract in normal dogs.

	Time after injection		0	10 min.	20 min.	35 min.	60 min.
Experiment No. 4 Dog 15 kg.	3.2 cc. Commercial Pituitary Extract	Glucose, mgm. %					
		CO_2, vols. %	47.2	21.5	24.7	30.8	40.9
Experiment No. 13 Dog 15 kg.	3.0 cc. "Pitocin"	Glucose, mgm. %	83	139	156	124	99
		CO_2, vols. %	51.7	38.5	43.8	45.4	48.5
Experiment No. 10 Dog 22 kg.	1.9 cc. "Vasopressin"	Glucose, mgm. %	87	178	167	148	106
		CO_2, vols. %	45.2	24.1	22.4	26.4	35.0

Pituitary extract has a wide vogue in the treatment of surgical shock. Since a considerable degree of acidosis is frequently a factor in this condition, the administration of pituitary extract is obviously contraindicated.

Further work on this problem is in progress.

4592

Effect of Thyroxin Upon Normal, Hypophysectomized, and Thyroidectomized Tadpoles.*

BENNET M. ALLEN.

From the University of California at Los Angeles.

The removal of the thyroid gland anlage and the buccal anlage of the hypophysis from amphibian larvae affords animals in which these glands have never functioned. In the first series *Rana aurora* tadpoles kept for over 4 months after the operation were placed in thyroxin solutions of 1-500,000; 1-1,000,000; and 1-2,000,000 concentration in tap water. In all cases there was a very rapid tendency to metamorphosis that proceeded with equal speed and to an equal degree in each of the groups, resulting in the appearance of all 4 limbs except in a few cases in which the right fore-limb failed to pierce the skin. The tail rapidly shrivelled and typical changes took place in the position of the eyes, the character of the mouth, the retrogression of the gills, and, all of the other features characteristic of thyroid induced metamorphosis. The rapidity of the changes of metamorphosis was to a slight degree inversely proportional to the concentration of the thyroxin solution, as we should expect; but some diversity in size of specimens precludes our attaching too great importance to this point.

Controls maintained during this time under identical conditions failed to show any tendency to metamorphosis. The largest had only the hind-limbs developed to the point of showing the knee-joints, and the fore-limbs had not appeared in any, nor was there any shortening of the tail or other evidence of metamorphosis. Because of these facts we may conclude that the thyroid gland and anterior lobe of the hypophysis in the normal specimens placed in thyroxin were not factors to be considered; probably because they had not yet become active to any significant degree.

This view is strengthened by the fact that the thyroidectomized and hypophysectomized tadpoles responded to the thyroxin to the same degree as the unoperated specimens.

From the earlier work upon thyroidectomized and hypophysectomized tadpoles it has been clear that these glands are not essen-

* This work was supported by a research grant of the University of California. The writer wishes further to acknowledge his deep indebtedness to Dr. Gregorio Del Amo for the privilege of enjoying the kindly hospitality of his research laboratory.

tial to development, until the appearance of the hind-limb buds. But beginning with the stage to which our specimens belong, they play an increasingly important rôle.

Table to Show the Influence of Thyroxin Solutions upon *Rana aurora* Tadpoles. Figures Refer to Averages Made up from Groups of 5

	Length at start	Length	Change in length
Thyroxin solution 1–500,000. 11 days in solution			
Thyroid	33 mm.	18 mm.	—15.0 mm.
Hypophysis	34 mm.	20 mm.	—13.5 mm.
Normal	32 mm.	18 mm.	—13.6 mm.
14 days in solution—1–1,000,000			
Thyroid	45 mm.	26 mm.	—19.2 mm.
Hypophysis	41 mm.	23 mm.	—18.0 mm.
Normal	33 mm.	17 mm.	—15.6 mm.
15 days in solution—1–2,000,000			
Thyroid	44 mm.	32 mm.	—12.2 mm.
Hypophysis	46 mm.	33 mm.	—13.0 mm.
Normal	39 mm.	27 mm.	—11.8 mm.
Control tadpoles not kept in thyroxin solution			
4 Normal	42 mm.	41 mm.	—1.0 mm.
2 Thyroidectomized	57 mm.	57 mm.	0.0 mm.
2 Hypophysectomized	37 mm.	38 mm.	+1.0 mm.

Large numbers of tadpoles of all these types were kept in the laboratory and none showed the characteristic thyroid changes. All of the specimens in thyroxin solutions showed them markedly and none of the 8 controls showed them in the slightest degree.

It may be contended that the concentration of thyroxin in the above experiment was not weak enough to demonstrate small differences in the response of these 3 classes of tadpoles, so an experiment was undertaken in which dilutions of 1:20,000,000 and 1:40,000,000 were employed. The number of thyroidectomized specimens was limited so that groups of 5 each were chosen, and the following averages represent surviving lots of from 3 to 5. Each lot of tadpoles was kept in 200 cc. of solution for 42 days. In no case was complete metamorphosis induced, although there was an approach to it in a few cases. Because of the slow development it is fair to say that the more extreme dilution is not far from the threshold of stimulation, but the effect is very marked as shown by a comparison with the controls.

An examination of the above table will show that hypophysectomized and thyroidectomized tadpoles respond to this very dilute thyroxin solution with a readiness fully comparable to that shown by the controls. In this case, hind leg length is taken as a criterion of the degree of metamorphosis rather than total length, because it is in many ways a more satisfactory one. The results are similar. Still greater dilutions and larger numbers of specimens might show

Table to Show the Influence of Very Dilute Thyroxin Solutions upon *Bufo halophilus* larvae. Measurements taken after 42 days.

	Total length	Trunk length	Hind leg length
Control in tap water			
	mm.	mm.	mm.
Normal	23.63	10.23	.77
Thyroidectomized	22.96	9.37	.85
Hypophysectomized	27.17	11.43	1.31
Thyroxin solution 1:40,000,000			
Normal	18.30	8.76	3.81
Thyroidectomized	30.47	12.77	5.63
Hypophysectomized	24.32	10.12	4.68
Thyroxin solution 1:20,000,000			
Normal	19.05	8.77	4.04
Thyroidectomized	26.15	10.60	4.65
Hypophysectomized	19.67	8.00	3.63

quantitative difference in response but the writer feels justified in concluding that (1) metamorphosis of thyroidectomized and hypophysectomized tadpoles is readily induced by extremely dilute solutions of thyroxin. (2) The early secretion of these glands does not render the tadpoles either more or less sensitive to thyroxin than the normal ones. (3) The presence of neither the hypophysis nor the thyroid gland is essential or even apparently conducive to the reaction of the tadpole to the thyroxin. Work done several years ago in feeding iodine to thyroidectomized and to hypophysectomized tadpoles demonstrated the fact that elemental iodine would produce metamorphosis when fed to thyroidectomized and hypophysectomized tadpoles. The present work tends to show that there is no difference in the speed of their response to thyroxin.

4593

Effect of Fatigue on Protein Consumption.

FREDERICK P. BROOKS. (Introduced by W. deB. MacNider.)

From the Laboratory of Physiological Chemistry, the Medical School, University of North Carolina, Chapel Hill.

Some recent papers on protein consumption and basal metabolism set forth the fact of regular weekly variations in urinary nitrogen excretion. Borgstrom and Bost[1] and Borgstrom, Hafkesbring and Bost[2] showed that in a series of analyses of urine collected over a period of months there was evidence of a weekly cycle of values.

[1] Borgstrom, P., and Bost, R. W., *Am. J. Physiol.*, 1926, lxxix, 229.

[2] Borgstrom, P., Hafkesbring, R., and Bost, R. W., *Am. J. Physiol.*, 1926, lxxix, 237, 245.

On certain days of every week high values were observed for one subject on the basis of fatigue and on the basis of muscular exercise for the other. The writer had observed a recurrent variation in some weekly values determined for a few individuals. In these cases it was difficult to explain the variation on the grounds of exercise but it appeared more likely that fatigue played the more important rôle. It seemed worth while to secure more evidence in support or refutation of this idea. It would be of interest in regard to certain values reported for students,[3, 4, 5] for if certain days of the week give high or low values and the data reported were obtained on these days, these reports would represent levels which might be well above or below the weekly average, and the protein habit of the individuals studied would be incorrectly represented.

If fatigue exerted an appreciable effect upon the protein consumption, this effect would be revealed in the daily urinary nitrogen excretion of medical students since this group lives under a rather strenuous and regular schedule. In this institution the schedule of the first year is rather full, allowing little time for outside activities, and few students take any regular exercise. It would be expected that a gradual increase in fatigue would be seen from Monday to Friday. From Friday night until Monday represents a period of relaxation and, in many instances, an unusual amount of muscular activity. If this condition of fatigue should exert an appreciable influence on the protein intake the weekly curve of urinary nitrogen excretion should show values above average for Saturday, Sunday, Monday and probably Tuesday, with low average values the remainder of the week. The curve would probably run from a maximum on Monday or Tuesday to a minimum on Friday with the intermediate days grading between. The general slope of the curve should be downward from the first to the last of the week. Curve 1 of Charts I and II represents this idea.

It was not to be expected that such a variable as urinary nitrogen on an uncontrolled diet would regularly give any such smooth curve as Curve I even if the effect existed as postulated. It would indeed be remarkable if a large percentage of all the cases studied should show conformity.

Experimental. In 1928 a group of students collected 24-hour urine specimens on one day a week for 7 weeks. A different day of each week was selected so that results were obtained representing

[3] Beard, H. A., *Am. J. Physiol.*, 1927, lxxxii, 577.

[4] Brooks, Frederick P., *Ibid.*, 1929, lxxxix, 403.

[5] Denis, W., and Borgstrom, P., *J. Biol. Chem.*, 1924, lxi, 109.

the daily excretion and at the same time representing the protein habit over a period of 2 months. On the whole the collections were trustworthy. The completeness of the collection was judged from specific gravity—volume relationships and the results were discarded where obvious error appeared. The analyses here reported were all made by the writer using the Gunning modification of the macro Kjeldahl method.

In Chart I are shown graphically certain of the results. These were selected because they represented complete 7-day collections, while those not reported were incomplete due to the closing of the term. The values represent the nitrogen from the urine collected on

CHART I.—*Daily Urinary Nitrogen Excretion in gm.*

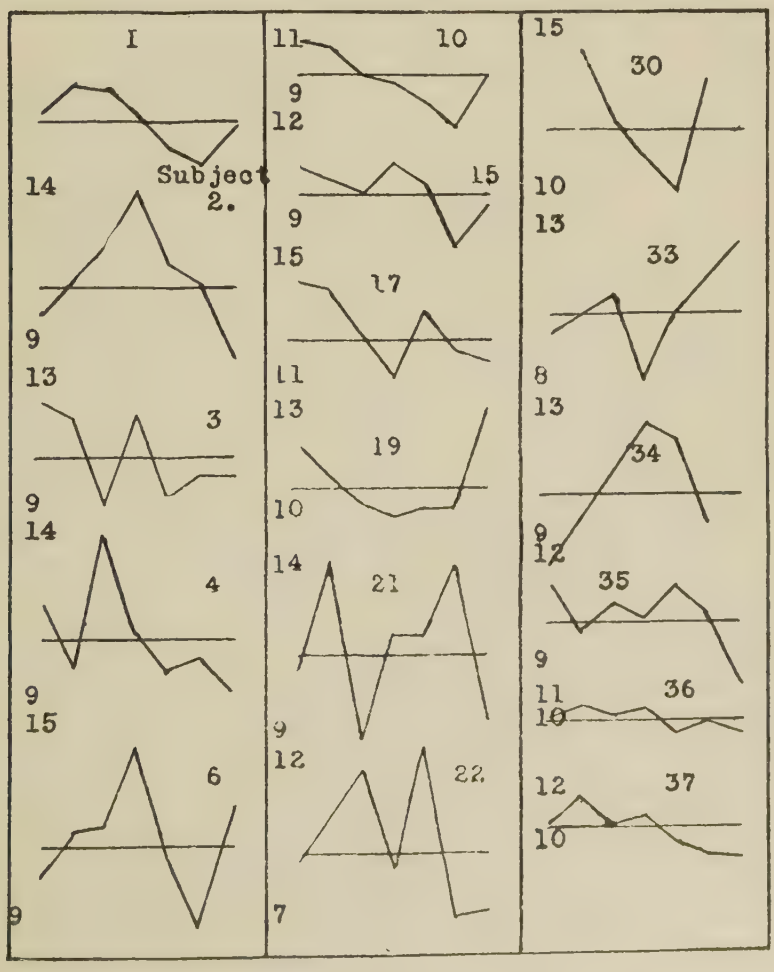


CHART II.—*Daily Urinary Nitrogen Excretion in gm.*

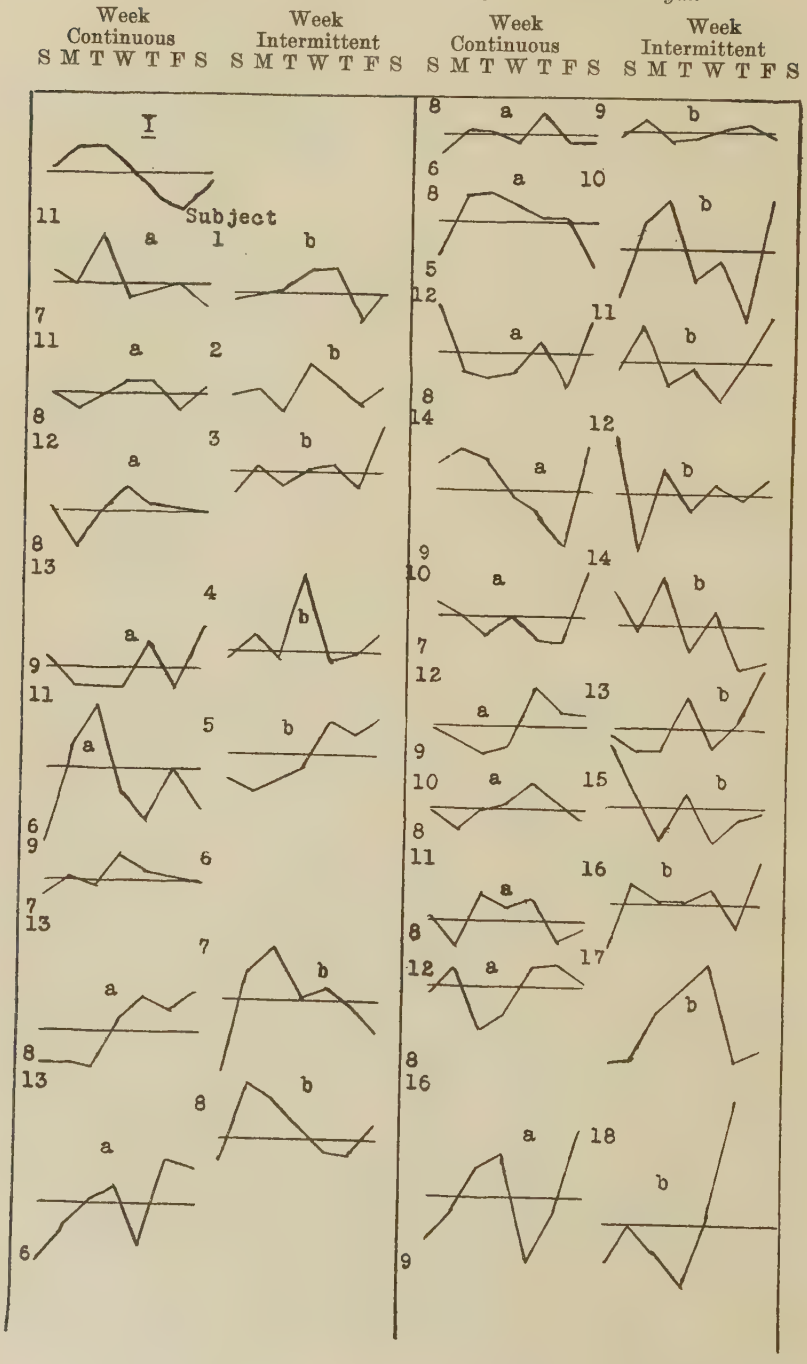
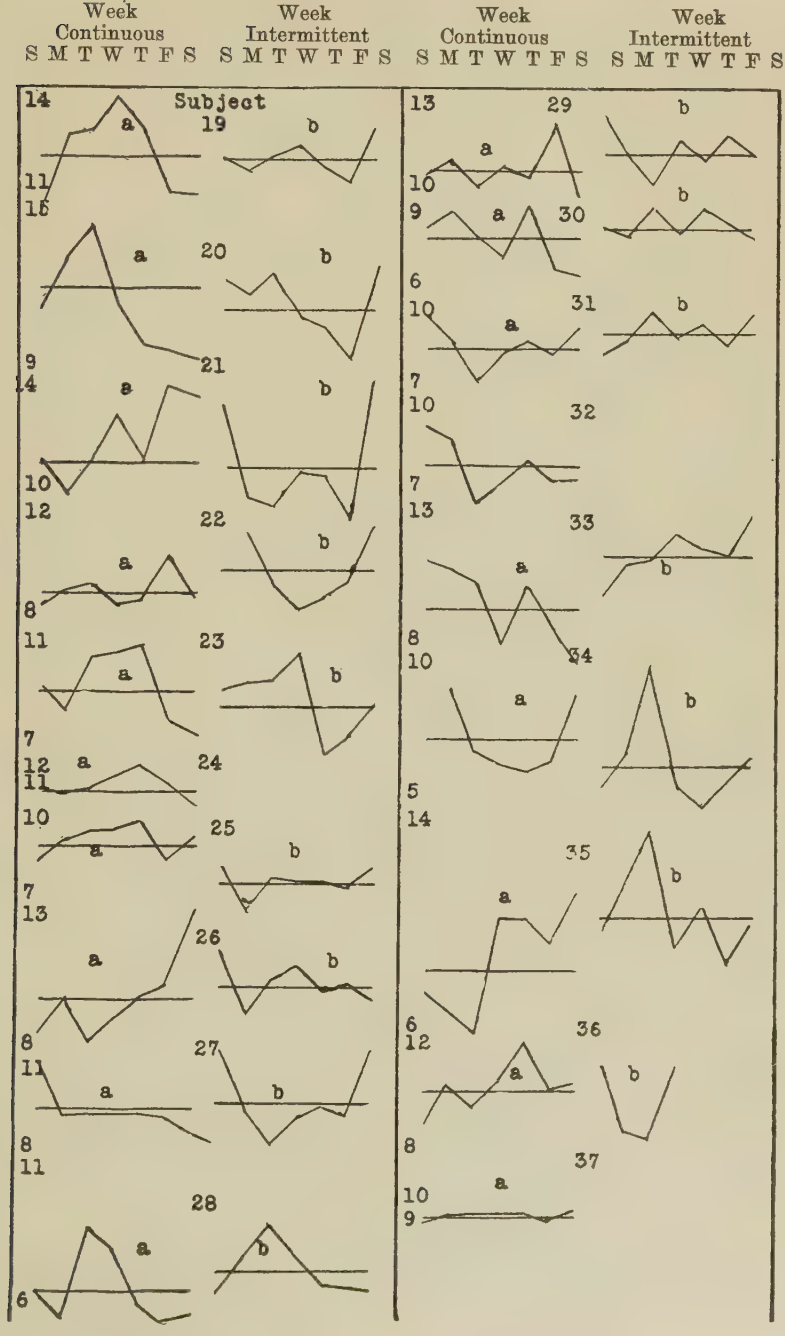


CHART II. *(Continued).*



the day designated. The specimens were completed on the morning of the day following the day labeled, *i. e.*, a Monday specimen is that collection made from 7 a. m. Monday until 7 a. m. Tuesday, etc. These data were collected during February and March of the year noted. The average value for the group, the per 70 kilo equivalent and the temperature of the period have been reported in a previous paper.[4]

In January and February, 1929, a second series of determinations was made from a group of 36 male medical students. The system of collection was the same for 2 months. Each student made a daily collection for one continuous week. The sample was analyzed and the completeness of the collection was checked by the specific gravity volume relationships of the various samples of each individual. The data for this series is shown in the plotted curves of Chart II.

Discussion. A study of Chart I shows the average of the group in fair agreement with Curve 1. Compare Curves 1 and 36 of Chart I. Curve 36 represents the average daily values for the entire group of 35 subjects, while Curve 37 represents the average of the curves shown in Chart I. These are both in fair agreement with Curve I, which is the postulated form of the curve which should show the fatigue effect under the described conditions. An analysis of the curves in Chart I is given below in Table I.

A study of the average values for Group II reveals a remarkably close agreement of all the days of the week and of the week of continuous collections with the week of intermittent collections over a period of 2 months. Curves 37 and 37b, Chart II, show the average daily values for the continuous and intermittent weeks respectively as almost horizontal lines. This would seem to indicate a failure of the protein consumption to respond to fatigue. It certainly indicates that with a sufficiently large group of subjects specimens collected on any day of the week will give the protein habit of the group accurately. From this it appears that the results of Borgstrom and Denis, Beard, and Brooks do represent the habits of the groups studied in so far as any fatigue effect would have vitiated them. Many of the variations observed show maximum values where minimum values were expected. But an analysis of the individual curves of Charts I and II gives more support to the idea of a fatigue effect than do the total averages of the groups.

These curves may be classified in 5 groups as follows: (1) Curves showing maxima toward the first of the week; (2) Curves showing maxima toward the last of the week; (3) Curves showing maxima

TABLE I.
Classification of Curves in Charts I and II.

Data	No. of Curves	Type 1	Type 2	Type 3	Type 4	Type 5
Chart I	16	9	1	2	2	2
Chart IIa	36	13	9	5	3	6
Chart IIb	32	11	3	3	4	11
Total	84	33	13	10	9	19
Percentage of each type		39.9	15.5	12	10.6	22.6

in the middle of the week; (4) Curves showing minima in the middle of the week; (5) Curves showing no tendency toward either maxima or minima. Type *1* is seen in Curve I. Type *2* is seen in Curve 4, Chart II. Type *3* is shown by Curve 2, Chart I. Curve 19, Chart I, represents type *4*. Type *5* is found in Curve 35, Chart I.

From this analysis it is evident that there is a preponderant number of curves of type *1* which certainly shows a strong suggestion of an effect which I have postulated, a fatigue effect. It is quite surprising that types *1* and *5* should make up nearly two-thirds of the total number. It is more surprising that the remainder of the curves which are divided into 3 equal size groups should be able to overcome the tendency shown by type *1* curves and bring the total average to a curve of the neutral type. This is explicable only on the basis of the very wide variations which are observed on some curves on all days of the week.

These weekly variations might be explained on the basis of menu if the group of subjects ate at the same place. However, the group patronized 12 different eating places and not more than 6 ate at the same place. Many ate at cafeterias and cafes where the menu was determined by their choice and financial condition.

The above results seem to indicate a response of protein consumption to fatigue in the case of medical students living on a rather regular schedule. They also show that any day of the week will be satisfactory for the collection of urine specimens for the determination of protein consumption provided a large group of subjects is used for obtaining the average. This apparent inconsistency, *viz.:* a tendency for the daily excretion to diminish in response to fatigue and a representative average for the group on any day of the week is explainable on the grounds of the great irregularity in the nitrogen excretion of certain individuals on each day.

Summary. 1. The results of approximately 105 weeks of urine collections by 70 individuals are given in terms of N excretion. 2. The daily values are plotted and it is found that there is a decided ten-

dency toward a decreasing daily excretion from Sunday to Saturday. 3. These results are interpreted as supporting the claim of a fatigue effect upon protein consumption. 4. It is shown that in spite of this tendency specimens collected on any day of the week may be used to give an accurate idea of the protein consumption of the group if the group is large enough.

4594

Source of Bioelectricity, Investigated by the Relation Between Stainability and Electric Charges in Tissues and Artificial Models.

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Little is known about the electrical action inside of living tissue. Its nature and cause can be elucidated to a certain degree by comparing stainability and electromotive forces.[1]

Numerous previous experiments have demonstrated the following relation between the stainability of tissues and bioelectric currents. Structures bearing a relatively negative charge are preferably stained by eosin and certain other acid dyes, while electrically positive struc-

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[1] It may be added that electrical potential differences must be present in tissue everywhere, *viz.*, at every phase boundary and also at any place where diffusion occurs. The electromotive property of the skin of plants is analyzed by its extraordinarily large and regular variations following changes in the concentration of the solutions in contact with it. This effect can *not* be reproduced by means of protein as has been maintained by J. Loeb. (Loeb, J., *Proteins and Colloidal Behavior*, 1922; Höber, *Zeitschr. physik chemic.*, 1924, cx, 142.) None of the values given by Loeb, Höber, and their collaborators amounts to more than one-fifth of the maximal effect of concentration obtainable in plant and animal tissue.

The so-called 'protein' effects demonstrated by Höber with various salts are, moreover, just water effects. His assertion that this of itself exclusively should explain bioelectricity is contradicted by numerous facts. With a *few selected* substances only the maximal effect of concentration can be reproduced. Among these substances is dried collodion. The electromotive action of this substance need not necessarily be explained as L. Michaelis (*Biochem. Zeitsch.*, 1925-26, nine papers; also *J. Gen. Physiol.*, 1927-29, eight papers) suggest, as due to pores of molecular dimensions which cause selective ionic permeability. This case does not necessarily require an entirely different theory from other similar cases.

tures seem to attract methylene blue and other staining bases, as expressed by the following scheme:

+ Saline solution	*basophilic* structure (stained by *methylene blue*)	*acidophilic* structure (stained by *eosin*)	saline solution —

G. W. Crile was the first to indicate a similar relation in his so-called Bipolar Theory, which is the expression of his finding that the stainability of tissue runs parallel to the electric potential of its current of injury.[2] Crile found that both stainability and potential are decreased in exhaustion, chronic poisoning, hemorrhage, shock, infection, etc. J. Gicklhorn, R. Kellar and others have offered definite experimental proofs of the above relation by studying the stainability and electromotive forces of plant tissues.[3]

Artificial systems have now been found which exhibit a relation between stainability and electromotive forces similar to that observed in living tissues. One such system is the following:

+ Saline solution pH = 7.4	layer of olive oil + oleic acid (with or without amine basophilic) I	layer of olive oil + amylamine (acidophilic) II	saline solution — pH = 7.4

When shaken with a mixture of methylene blue and eosin (Wright's stain or Pianese's mixture), I stains like basophilic tissue; for example, the nucleus; II stains like acidophilic tissue; for example, the cytoplasm.

Various other oils may be used, cocoanut oil, tributyrin, or triacetin; and also various esters, ethyloleate, ethylcaproate, ethylbutyrate, ethylacetate or amylacetate, cetylacetate, etc. The use of lecithin or cholesterol as a neutral fatty solvent offers considerable experimental difficulties, but it has been observed, at least qualitatively, that similar electric effects can be produced by the addition of oleic acid or amine. Amylalcohol, or other higher alcohols with or without the addition of hydrocarbons, can also be used. It appears to be the rule in a system of the kind described that any neutral water-immiscible solvent becomes electrically positive by the addition of an oil-soluble acid, and electrically negative by the addition

[2] Crile's original publication on the "Bipolar Theory" fails to contain direct measurements of potential differences; these were made a short time later by him and his collaborators and the results were found to agree with the theory as stated in his book. (Crile, *Arch. Surgery*, 1921-25, six articles.)

[3] R. Keller has suggested that this relation should exist and has induced J. Gicklhorn to perform the measurements. (Keller, *Elektr. in der Zelle*, 1925; Gicklhorn and Umrath, *Protoplasma*, 1928, iv, 228.)

of an oil-soluble base—positivity being associated with basophilic staining and negativity with acidophilic staining.

From the standpoint of physical chemistry this finding must be expected, since *phase boundary potentials* are located at the junction between any of these water-immiscible layers and the aqueous solutions in contact with them. These potential differences must be differentiated in the direction actually observed on account of a soap content of the oleic acid layer; which leads to an excess of Na ions in that layer. On the other hand the presence of amine has no such action. The amine, on the contrary, combines with any oil-soluble acid constituents which may be present, *e. g.*, oleic acid from saponification of olive oil—and thus tends to diminish the concentration of the Na+ ions in the olive oil. Consequently the cell arrangement, mentioned above, is really a concentration cell in regard to Na+ ions:

	Na+ in water	Na+ in oil	Na+ in oil	Na+ in water
+	about 0.1 molecular	high (Na oleat)	low (amine)	about 0.1 molecular —

According to well known laws, this system must have an e.m.f. in the direction observed.†

4595

Composition of Bone IX. Equilibration of Serum with $CaHPO_4$.

M. J. SHEAR AND BENJAMIN KRAMER.

From the Pediatric Research Laboratory, The Jewish Hospital of Brooklyn, N. Y.

Because of the complications introduced by the presence of proteins in serum, we studied first the solubility equilibria in protein-free solutions. We[1] found that, at the pH of serum, solutions with the inorganic composition of ricketic serum are markedly undersaturated with respect to $CaHPO_4$. Solutions with the calcium and phosphorus content of normal blood serum are slightly undersaturated; it is only solutions which have Ca x P products greater than about 50 which are supersaturated with respect to $CaHPO_4$.

Since part of the calcium in serum is bound to protein, it was

† The writer wishes to express his appreciation to Dr. G. W. Crile of the Cleveland Clinic Foundation for his kind interest in this work.

[1] Shear, M. J., Washburn, M., and Kramer, B., *J. Biol. Chem.*, 1929, lxxxiii, 697.

predicated that ricketic serum is undersaturated with respect to $CaHPO_4$; normal serum should be slightly undersaturated with respect to this substance.

This theory was tested by shaking blood sera for one hour at 38° with crystalline $CaHPO_4$. As a result of the equilibration, the phosphorus increased in all cases; in some cases both calcium and phosphorus increased. In every case equilibration produced an increase in the Ca x P product and in the $[Ca] \times [HPO_4'']$ product. In the sera as drawn, the Ca x P products ranged from 35 to 85, and the $[Ca] \times [HPO_4'']$ products ranged from 2.4×10^{-6} to 5.7×10^{-6}. The final Ca x P products ranged from 74 to 88; the final $[Ca] \times [HPO_4'']$ products ranged from 5.3×10^{-6} to 6.5×10^{-6}.

The sera used were obtained from a young calf, young lambs and from human beings. All of them were found to be undersaturated with respect to $CaHPO_4$. In inorganic solutions of the same ionic strength, $[Ca^{++}] \times [HPO_4'']$ had been found to be 3.4×10^{-6} at equilibrium. The equilibrium values of $[Ca] \times [HPO_4'']$ in sera are greater than this, as was expected since, in serum, [Ca] is greater than $[Ca^{++}]$. The values obtained may therefore be utilized in calculating the amount of "bound" calcium and ionized calcium in serum.

4596

Irreversible Character of the Late Changes after Hepatectomy.

PHILIP D. MCMASTER AND D. R. DRURY.

From the Rockefeller Institute, New York City.

We have endeavored to learn whether rabbits manifesting the symptoms characteristic of the advanced stage of liver insufficiency[1,2] can be clinically improved by the circulation of their blood through the livers of healthy animals or by cross transfusion with normal rabbits.

"Liver transfusion." In an initial series of 14 experiments, rabbits, hepatectomized under ether and with cannulae placed in the left carotid artery and left jugular vein, were given sufficient glucose to maintain the blood sugar level well above normal. Fourteen to 24 hours later, when the characteristic signs of advanced hepatic insufficiency in the rabbit[1, 2] had appeared, the portal vein and vena

[1] Drury, D. R., *J. Exp. Med.*, 1929, xlix, 759.

[2] McMaster, Philip D., and Drury, D. R., *J. Exp. Med.*, 1929, xlix, 745.

cava connecting with the liver of a healthy animal were rapidly cannulated, the hepatic artery was tied and the organ was removed. It was submerged at once in a bath of paraffin oil at 40° C., and connected with the circulation of the liverless animal by means of the cannulae already present in the carotid artery and jugular vein of the latter. In this way the circulating blood of the liverless animal was passed for as long as an hour through the freshly removed liver of the healthy rabbit.

Of 14 experiments 4 were carried to completion without lapses of technique which might render the findings questionable. In none of the 14 did clinical improvement of the liverless animals take place though the "transfused" liver was actively functioning as shown by a copious formation of bile.

Cross transfusions. The effect was next studied of cross transfusions between normal rabbits and liverless ones showing the signs of advanced hepatic insufficiency. Of 15 such experiments 5 were completed without technical lapses. In 2 of these latter the blood, leaving by cannula in the proximal end of a carotid artery of each animal, entered the circulation of the other one through a cannula placed in the distal end of its carotid. In the 3 remaining instances, cross transfusion was performed from the carotid artery of the one animal to the jugular vein of the other. The rabbits receiving the blood from hepatectomized individuals in these ways over periods up to an hour and a half showed no ill effects; but, on the other hand, the symptoms of liver deprivation were not ameliorated nor death in consequence of it deferred. This held true in all 15 cases.

From the findings here reported, the late changes after liver deprivation, those leading to death, would appear to be irreversible.

4597

Glucose Requirement of Hepatectomized Rabbits and Its Relation to Lactic Acid Production.

D. R. DRURY AND PHILIP D. MCMASTER.

From the Rockefeller Institute for Medical Research, New York City.

We have previously reported[1] on the minimal glucose requirements of the hepatectomized rabbit during the first 6 to 8 hours after operation. During that period, a constant intravenous injec-

[1] Drury, D. R., and McMaster, P. D., *J. Exp. Med.*, 1929, xlix, 765.

tion at the rate of 125 mg. of glucose per kilo per hour will maintain a normal blood sugar level. The oxygen consumption of these animals indicated that they would have required 500 mg. glucose per kilo per hour if their energy requirements had been entirely supplied by glucose. The difference between these two figures represents mainly the amount of fat oxidized, expressed as its glucose equivalent. The respiration quotients of approximately 0.77, in these animals, bears out this assumption.

The blood sugar level can be maintained in liverless animals for periods up to 15 hours after operation by the injection of glucose at the rate above mentioned. Eventually, however, the blood sugar begins to decrease and the amount of glucose must be increased to bring it back to normal. Thereafter added increases must be made at least every hour to maintain the blood sugar level. Finally, a rate of about 500 mg. glucose per kilo per hour is arrived at, which is the maximum amount ever required and one on which the normal sugar level can be maintained until the animals die.

It is at the time when the first increase in the quantity of glucose is required that the symptoms of the so-called second stage after hepatectomy develop. At its beginning there is restlessness and irascibility, then blindness and ataxia, followed by extreme weakness, and coma. We noticed that the lactic acid content of the blood became higher as the second stage progressed and it seemed pertinent to ascertain whether the increase in the glucose requirement of the liverless animal is due to an incomplete utilization of glucose by the organism, with the production of lactic acid.

It has been found that when both the liver and kidneys of a rabbit are removed the blood lactic acid drops slowly during the first 8 to 15 hours from the rather high level consequent on etherization. Then, as the second stage develops, the lactic acid begins to mount again and rises about 70 mg. per 100 cc. on the average during this period, the maximum rise noted being 150 mg. The intravenous injection of 100 mg. per kilo of dextro-lactic acid (injected as calcium lactate) into the hepatectomized, nephrectomized rabbit raises the blood lactic acid about 50 mg. per 100 cc. This suggests that the rise of 70 mg. in the blood lactic acid during the second stage after hepatectomy would be caused by production of about 150 mg. lactic acid. This increase takes place over a period of from 5 to 8 hours, giving an average lactic acid production in the animal of some 30 mg. per kilo per hour. Since one mg. lactic acid results from the breakdown of one mg. glucose, the production of this small amount of lactic acid cannot be held to account for the large increase of

glucose consumption, which amounts during the last hours of life to 375 mg. per kilo per hour. The explanation of the increase must be sought in other directions, either in the incomplete breakdown of glucose to form other products than lactic acid, or in an inability of the animal to utilize fat as at first. There is no evidence of an increased metabolic rate during this period.

4598

The Relation of pH Value of Medium to Selective Bacteriostatic Action of Dyes.*

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From the Laboratory of Experimental Therapeutics, Cornell Medical College.

Several years ago experiments were carried out in this laboratory to determine whether changes in pH value of the medium with which the experiments were conducted would alter the character of the selective bacteriostatic activity of gentian violet. In these experiments, the results of which were never published, a series of divided plates with the following pH values were planted with *B. coli, B. anthracis, Staphylococcus* and *B. prodigiosus:* 5.4, 6.4, 6.6, 7.4, 7.6, 7.8, 8.8 and 9.3. The upper halves of the plates contained gentian violet in a strength of 1 to 200,000. On all the plates from pH 6.4 to pH 9.3 selective action of the dye took place exactly as on media of pH 7.2; growth of the Gram positives was inhibited, growth of Gram negatives was unaffected. At pH 5.4 no growth of any organism occurred even on the plain agar. Means were not then at hand for buffering the media in the alkaline range beyond 9.3 but in plates made of media to which large amounts of alkali had been added, certainly sufficient to give a pH well beyond 10, no growth occurred even on the plain agar. The conclusion was reached that, within the range of growth, pH of media was not a factor in determining the character of selective action of gentian violet.

The recent publication of Dubos[1] in which the suggestion is made that some of the inhibitory dyes owe their power of inhibition to the fact that they poise the media at an oxidation potential outside the range in which the inhibited organisms can grow, made it seem wise to repeat our earlier experiments. Divided agar plates, the

* Work aided financially by the Chemical Foundation.

[1] Dubos, Réné, *J. Exp. Med.*, 1929, xlix, 575.

upper halves containing gentian violet 1 to 200,000, were, therefore, made of media at the following pH values: 3.6, 3.8, 4.2, 4.4, 4.6, 4.8, 5.0, 5.2, 5.4, 5.6, 5.8, 6.0, 6.2, 8.5, 8.8, 9.3, 9.6, 10, 10.4, 11, 11.5, 12, 12.3, 12.7. To obtain the acid pH's HCl was added; to obtain the alkaline, sodium hydroxide. For the higher alkaline range M/10 CO_2 free sodium hydroxide and M/10 glycin solution were used as buffers.

In the acid range, at pH 5.2 selective action was as usual; at pH 5.0 *B. anthracis* failed to grow on the plain agar; at pH 4.6 there was no growth of any organism on either side of the plate. (See Fig. 1, a, b, c.) In the alkaline range selective action at pH 10 was as usual, beyond which point *Staphylococcus* and *B. anthracis* began to grow on the gentian violet side. (Fig. 1, d and e.) Since the dye had been obviously changed by the alkali, little significance could be attached to this fact. At 12.7 the selective action was as shown in Fig. 1, f. If plates were made of media to which still more alkali had been added (their pH could not be measured, but it was much higher than 12.7) no growth at all occurred on either side of the plate. (Fig. 1, g.)

The experiments were repeated in broth where the results were similar in principle, though the pH values at which growth failed to occur were slightly different.

These experiments seem to show that pH value of the media, if a factor at all, must be an insignificant factor in the selective

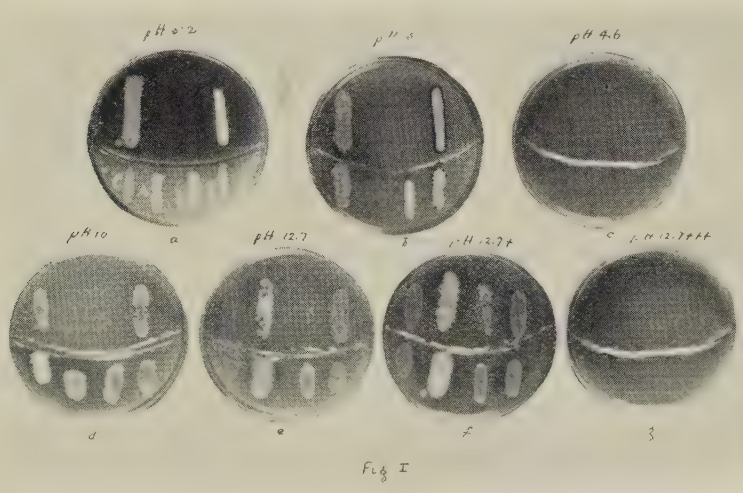
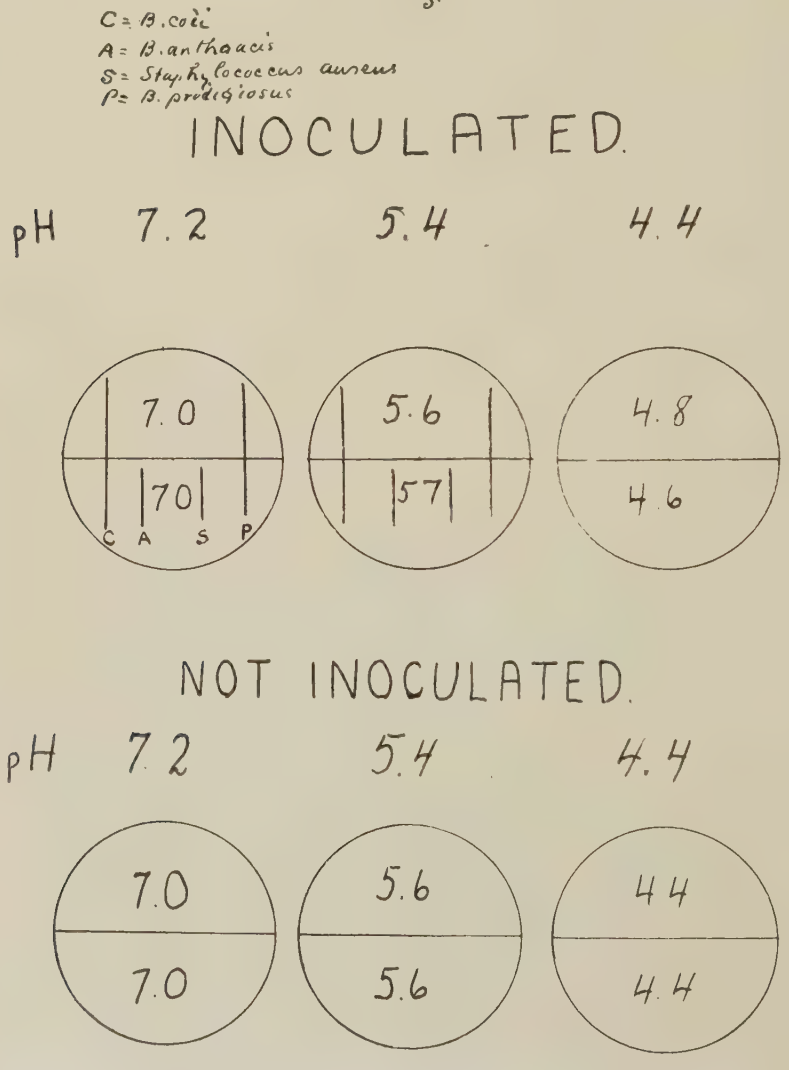


Fig. 1.

C = *B. coli*. A = *B. anthracis*. S = *Staphylococcus aureus*. P = *B. prodigiosus*.

bacteriostatic activity of gentian violet. It was thought that perhaps an error might have been made in drawing this conclusion, since there was a chance that the pH of the media used might have been changed by the bacterial growth or by the exposure of the plates in the incubator and, therefore, have been different at the moment the selective bacteriostasis took place from what it was when the plates were inoculated. Two series of 3 divided plates each were, therefore, made at pH's: 7.2, 5.4 and 4.4. Three of these were inoculated with *B. coli, B. anthracis, Staphylococcus* and *B.*

Fig. II



prodigiosus; while 3 others were put in the incubator without inoculation. After 24 hours the plates were removed. No growth at all had occurred on the 4.4 plate; on the other two, selective action had occurred as usual (see Fig. 2). The agars from each half of the plates were then carefully removed and their pH's taken. The results are recorded in Fig. 2. The pH of the dye containing agar was in every plate almost identical with that of the plain agar.

4599

The Use of Equations of the n-th Order to Describe the Action of Simple Haemolysins.

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From Washington Square College, New York University.

In all recent work concerned with the fitting of formulae to curves obtained for the action of the simple haemolysins it has been assumed that the "fundamental reaction" between the cells and the lysin is one in which the latter combines with some component (probably protein) in the membrane of the former, thus forming a new compound as the result of the formation of which the integrity of the cell is destroyed. Thus, the quantity of the cell component, S, destroyed, is proportional to the quantity of lysin, x, used up in the system, and the velocity of the reaction is given by

$$(1) \quad dx/dt = k\,(c-x)$$

whence

$$(2) \quad t = \frac{1}{k} \log \frac{c}{c-x}$$

where c is the initial quantity of lysin (in milligrams), where t is the time required to produce lysis of an arbitrary number of red cells, and where S is large compared to c. Since it is assumed that the complete lysis of n cells corresponds to the utilization of a constant quantity of lysin, we obtain, by putting x = const., and varying c in (2), a relation between the time for complete lysis of n cells and c, the initial concentration of lysin; when plotted, this relation gives the "time-dilution curve" for any particular lysin. If we are concerned with the number of cells, N, haemolysed from moment to moment by a particular concentration of lysin, from the beginning

of the reaction until its completion, we solve (2) simultaneously with

$$(3) \quad N = N_0 \int_0^x e^{-b^2x^2} dx$$

and obtain the S-shaped "percentage haemolysis curves".

For certain haemolysins under certain conditions, these expressions describe the experimental results excellently. Recently, however, we have examined the action of several simple lysins over very much longer periods than previously, observing the time-dilution and percentage haemolysis curves over periods as long as 300 minutes and as short as 6 seconds. When observations are extended in this way, it is plain that the above expressions require some modification, especially in 2 respects: (i) In the time-dilution curves the high concentrations of lysin produce haemolysis more rapidly than indicated by (2), and the low concentrations of lysin produce complete lysis after long times (100 to 300 minutes) when, according to expression (2), they should never produce complete haemolysis at all. Both these discrepancies were pointed out when the expressions given above were proposed as first approximations. (ii) The percentage haemolysis curves obtained in experiments with which low concentrations of lysin are used show far greater skewness (expressed by the ratio of the time required for 50% haemolysis to that required for 100% haemolysis) than the simultaneous solution of (2) and (3) provides for. This discrepancy is not easily detected, for technical reasons, and has hitherto escaped observation.

Suppose, however that the lysins concerned (saponin, the soaps, the bile salts, etc.) do not exist in a perfectly dispersed state, but that they exist in the form of aggregates of molecules of varying sizes, as may be expected from their semi-colloidal nature. Then an aggregate of 1, 2, 3 . . . molecules may react with each molecule of the cell component, S, and if each such molecule of S requires the interaction of a number of lysin molecules (say 6) it may obtain them by 6 additions of aggregates of 1, 3 additions of aggregates of 2, 2 additions of aggregates of 3, one addition of an aggregate of 6, or any one of a number of combinations of these possibilities. If, for example, the lysin existed in aggregates of 6, then the expression

$$dx/dt = k\,(c-x)$$

would describe the velocity of the reaction as in (1), but if it existed in aggregates of 3 only, then

$$dx/dt = k\,(c-x)^2$$

would give the velocity.

Thus, in general, if the lysin were to exist in aggregates of varying numbers we should have the velocity given by

$$(4) \quad dx/dt = k\,(c-x)^n$$

whence, if $1/p = n$,

$$(5) \quad kt = \frac{p}{p-1}\left\{c^{\frac{p-1}{n}} - (c-x)^{\frac{p-1}{n}}\right\}$$

in which n would be a measure of the mean state of aggregation of the lysin together with the mean number of combining molecules, and would have a meaning somewhat similar to that of the index n in Hill's equation for the dissociation of oxyhaemoglobin. The value of n in (4), moreover, will in general be greater than unity, and will not, as a rule, be a simple integer.

An expression such as (5) has been found experimentally to describe the action of all of the simple haemolysins examined. The fit of the calculated curves to the theoretical points is almost perfect and greatly superior to the fit obtained with the formulae used hitherto. As might be expected from the nature of the hypothesis on which the formulae are based, different values for n are found for different lysins under the same conditions, *e. g.,* for saponin $n = 2$; for sodium taurocholate $n = 1.8$; and for sodium oleate $n = 1.1$ (approximately, at 25°, with human red cells). Further, these values are altered by changes in temperature, electrolyte content, etc., which may be easily imagined to change the state of aggregation of the lysins, and although we do not at present consider the expressions given to have much more than empirical significance, such variations in n suggest that the hypothesis above is sound. It must be observed, however, that a similar formula could be arrived at by assuming that the molecules of the cell component react in various numbers with lysin molecules, and that a combination of both assumptions may ultimately be necessary.

It is of considerable interest that expression (5), together with (3), also describes the kinetics of certain complex haemolytic systems, *e. g.,* of complement-amboceptor systems. Thus, if the quantity of amboceptor is constant, (5) describes the relation between the time required for complete lysis and the concentration of complement c, provided that certain technical precautions are observed, while the combination of (5) and (3) gives the percentage haemolysis curves. In this system the value of n is usually about 1.3. The fact that these expressions apply to this form of lysis lends support to the views of Eagle and Brewer, *i. e.,* that the complement acts as a lysin and that the amboceptor merely "mobilizes" it at the cell surface.

4600

Electrodialysis as a Means of Characterizing Ampholytes.

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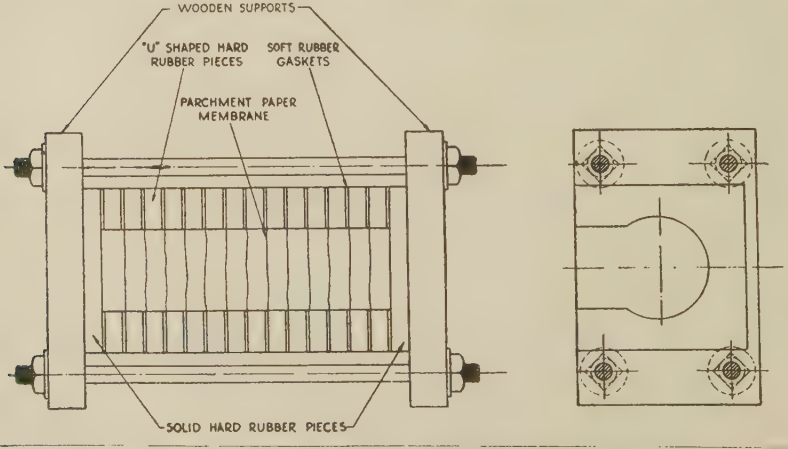
In the course of our studies of water soluble vitamins and bioses we have had frequent occasion during the past 5 years to enquire whether the physiological activity of an extract of a foodstuff was due to a single substance or a plurality of substances. Information was also often needed as to whether the substances involved were colloidal or crystalloidal, and if the latter, whether acidic, basic, amphoteric or non-electrolytic. This paper describes a method which often suffices to secure the answer to these questions by a single simple experiment, thus laying a good foundation for any attempt at isolation. The method also indicates the approximate isoelectric point of the substance in case it proves to be an ampholyte, a fact which is often of great service in suggesting the proper choice of precipitants. Kerr[1] has cited an instance in which the method was of use, namely in detecting β bios.

The method consists of electro-dialysing the solution in question in a multiple compartment cell. A convenient form of such a cell is illustrated in Fig. 1. After electrolysis the contents of the compartments are separately removed, their H^+ ion concentrations are determined and the solutions are assayed for the constituents of interest by chemical means if available, or by physiological test such as an



[1] Kerr, Ralph W., PROC. SOC. EXP. BIOL. AND MED., 1928, XXV, 344.

animal feeding experiment. If the substance sought is an ampholyte it may be concluded that the pH of that portion of the solution which contains the substance in maximum concentration approximates the isoelectric point of the substance. For at any pH greater or less than that of the isoelectric point, the substance will be ionized more strongly as a base than as an acid or vice versa and will therefore migrate toward the region at which ionization as acid and as base are equal, namely the region of its isoelectric point.

In order to test the validity of this reasoning we have electrolysed in a 12 compartment cell a solution composed as follows:

Quinine Sulphate (saturated sol.)	.346 gm.
Anthranilic Acid	.685 gm.
Valine	.585 gm.
Water to make 250 cc.	

These substances were chosen as representing a considerable range from acid to base and as being subject to ready analysis. The solution was apportioned equally among the compartments except in the case of the two terminal compartments which were of small volume, sufficient only to accommodate the electrodes. Electrolysis was carried out with platinum gauze electrodes with 110 volts, using a suitable resistance and milliammeter in series to avoid excessive current density (*i. e.*, over 2 milliamperes per sq. cm.) and consequent heating. As the resistance of the cell rose the line resistance was reduced and finally removed altogether. After 60 hours the current had dropped from 40 milliamperes to about 13 milliamperes, corresponding to a current density of 0.65 milliamperes per square cm. of cross section of liquid path. The potential drop from the cathode to the next adjoining compartment (No. 2) was 60 volts, and that from No. 2 to No. 3 was about 25 volts. Through the rest of the system the potential drop from cell to cell varied from 1 to 5 volts.

The solutions were removed and the H^+ concentration was determined colorimetrically in each. Sulphuric acid was determined by titration with barium hydroxide. Quinine was determined by extracting each solution with ether after rendering alkaline with excess barium hydroxide. After quantitative removal of barium as sulphate, the solutions were evaporated to dryness and the residues were extracted with ether, which dissolves free anthranilic acid but not free valine. Finally the valine was roughly determined as the water soluble portion of the ether insoluble residues.

The results are shown in Table I. It is to be noted that the SO_4 ion does not extend beyond the fourth cell from the anode and the quinine is confined to the cathode compartment in which it precipi-

TABLE I.

Composition and Characteristics of the Contents of 12 Compartments After Electrodialysis.

Compartment No.	Volume of liquid cc.	pH	Quinine + 3 H_2O mg.	SO_4 mg.	Valine mg.	Anthranilic acid mg.
1(Cathode)	5	8.6	147	0	39	10
2	24	5.4	Trace	0	196	2
3	24	4.2	0	0	140	25
4	23	3.8	0	0	82	52
5	23	3.7	0	0	55	75
6	22	3.5	0	0	26	75
7	20	3.5	0	0	22	85
8	19	3.4	0	0	12	66
9	14	3.3	0	20	*17	37
10	12	3.0	0	93	*25	*27
11	13	<2.8	0	160	*25	*27
12	5	<2.8	0	30	* 8	* 4

*Material was gummy, probably representing in part products of electrochemical decomposition reactions. The regions of maximum concentration were conspicuously the regions of most complete and perfect crystallization and freedom from color. The residues at the anode end were highly colored.

tated copiously. The maximum concentrations of valine and anthranilic acid respectively lie near the points where the H^+ concentrations approach those of pure solutions of each of the 2 substances, as determined by us in an independent experiment, *viz.*, pH 7.2 and pH 3.8 respectively. Electrokinetic redistribution of water took place during electrolysis, as is indicated by volume of solutions in Table I, but this does not account for the conspicuous concentration of each constituent.

This method is useful for the purification of ampholytes. The valine used in this experiment was obtained from Eastman. It was found by electrolysis to contain a considerable amount of a component of greater acidity than valine. This was removed by electrolysis in the same apparatus and the residual valine recovered by evaporation and recrystallization for use as above described. The anthranilic acid was conveniently purified by sublimation.

The above method applied to yeast extract has served to confirm certain suspicions which we have long entertained about the multipartite nature of what has been called vitamin B. Unfortunately the 2 factors which can be recognized most definitely by our present feeding technique migrate toward the alkaline region where they undergo fairly rapid decomposition, so that high concentrations of them cannot be obtained by this means. These factors are the antineuritic vitamin and the one which we have heretofore designated only as "the third factor".[2]

[2] Williams, R. R., and Waterman, R. E., *J. Biol. Chem.*, 1928, lxxviii, 311.

Grateful acknowledgment is made to the Carnegie Institution of Washington for financial support of this work.

4601

Further Consideration of Transmissibility of Human Upper Respiratory Infections (Common Cold) to the Ape.

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In a previous communication[1] we have reported the suitability of the anthropoid ape as an experimental animal for the study of the upper respiratory tract infections usually grouped under the term "common cold".

We showed (1) that the upper respiratory flora of these animals during periods of normal health very closely resembles that of humans, and (2) that these animals are extremely susceptible to "colds" when exposed to humans suffering from such infections and that the clinical manifestations of these infections in the ape are more or less identical with those observed in human beings similarly affected.

Further, in an effort to ascertain the possibility of communicating to anthropoids, by means of a filterable agent, upper respiratory infections comparable to the human cold, it was shown that filtered nasal washings obtained from humans suffering with typical colds when injected intranasally into apes produced typical colds in about half of the instances attempted. In all positive experiments Gram-negative anaerobes of the type described by Olitsky and Gates were cultivated. However, no etiological significance was assigned to these organisms.

The importance of control experiments was recognized and early in the above investigations, plain broth and heated filtrate intranasal inoculations were carried out but were soon given up as inadequate. It was felt that it would be of more value to use for controls filtered nasal washings obtained from humans who were not suffering from colds. However, in view of the difficulty of excluding, with any degree of certainty, carriers of the active agent

[1] Dochez, A. R., Shibley, G. S., and Mills, K. C., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 562.

during the time that the transmission experiments were being performed (October to March) it was deemed advisable to postpone this phase of the study until an inter-epidemic period.

These control experiments were carried out in June and July of this year. During these months "colds" were at a minimum. The apes were taken in turn and were placed in quarantine for periods varying from 4 to 19 days and were then inoculated intranasally with filtered nasal washings. The procedure which had been used for the transmission experiments of the winter[1] was carefully followed throughout. As a source of nasal washings, healthy individuals, who had had no colds nor sequelae for at least 3 to 4 months and who had had no known exposure to current colds, were used.

The results of these experiments are striking in that they were absolutely negative. No change whatever was noted in the health of the animals either constitutionally or with respect to their upper respiratory tracts. In contrast with the changes noted in the positive transmission experiments there were no changes noted from the characteristic normal flora of their noses and throats. There was an entire absence of even small amounts of nasal mucous discharge following inoculation. In the light of these findings we have been led to consider as positive transmission experiments certain of our earlier results in which the symptoms and signs were rather inconspicuous and which we considered as doubtful.

It is very important to note that Gram-negative anaerobes of the type described by Olitsky and Gates were cultivated from these control filtered nasal washings in 75% of cases. Although this would seem to provide strong evidence against the probability that these organisms play an etiological rôle in the production of the common cold, it may be possible that there exists a specific type of these anaerobes that is a factor. This aspect of the problem is still under investigation.

The findings herewith presented taken in conjunction with the results of the transmission experiments already reported, seem to lead rather strongly to the assumption that the type of upper respiratory tract infection under consideration is caused by filterable virus.

4602

A Rapid Precipitation Test for Syphilis.

L. ROSENTHAL. (Introduced by J. Bronfenbrenner.)

From the Laboratories of United Israel Zion Hospital, Brooklyn, N. Y.

Ingredients: This test unlike the other existing precipitation tests for syphilis requires no dilution of the serum and antigen either before or after mixing them. Therefore the test is dealing with only two ingredients: serum and antigen.

Serum: The serum is obtained and inactivated in the usual way. As only small amounts of serum are needed it may be sufficient to secure the blood from the finger.

Antigen: Since the work of Sachs[1] in 1911, it has been a well established fact that the addition of cholesterin to the alcoholic beef heart antigen increases the sensitiveness of the latter in complement fixation and precipitation tests for syphilis. The solubility of cholesterin in alcohol is limited and therefore the cholesterin content in the alcoholic antigen cannot go beyond a certain degree (about 0.8%). It was thought that by using better solvents it would be possible to increase the cholesterin content of antigen. After several experiments acetone (at 37°) was found to be satisfactory for that purpose and the antigen was prepared by adding 2% solution of cholesterin in acetone to an equal volume of alcoholic beef heart extract. This extract is obtained by adding 5 cc. of alcohol (95%) for every gram of beef heart muscle powder from which the ether soluble substances were previously removed by ether extraction. The principle of preliminary ether extraction introduced by Neyman and Gager[2] and adopted by Kahn,[3] Meinicke,[4] *et al.*, was found to be of advantage also in this test. It is advisable to keep in stock separately the alcoholic extract and cholesterin solution and to prepare mixtures sufficient for only one week's need. If cholesterin crystals precipitate out the solution is placed in an incubator at 37° in order to dissolve them. If a turbidity occurs during the mixing of the cholesterin solution and the alcoholic extract, it is necessary to centrifugalize the mixture and use the supernatant clear fluid. In order to make the final results more conspicuous for reading 0.05 methylene-blue powder is added to 10 cc. of the cholesterinized antigen.

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1 Sachs, *Berl. klin. Wchn.*, 1911, xlviii, 2066.

2 Neyman and Gager, *J. Immunol.*, 1917, ii, 573.

3 Kahn, "Serum Diagnosis of Syphilis by Precipitation," Baltimore, 1925.

4 Meinicke, *Deut. med. Woch.*, 1922, xlviii, 384.

Glassware: For every test are needed: 1. A hollow ground slide; 2. A set of two capillary pipettes: one for the serum and the other for the antigen. In order to have the same caliber of the capillary stem for serum and for antigen both pipettes are drawn from the one piece of glass tubing. As a standard, we are using pipettes which contain 8 drops to 0.1 cc. of serum. 3. A glass rod.

Performance of the test: Four drops of serum are placed in the cavity of the hollow ground slide, and one drop of antigen is floated on the surface of the serum and allowed to stay for 2 minutes. Then the serum and antigen are mixed thoroughly with a glass rod, the slide is gently tilted and rocked for one-half minute and then examined. If the room temperature is low it is recommended to use serum and antigen which have been warmed in the incubator at 37° for 15 minutes. It must be borne in mind that the ratio of 4 drops serum to 1 drop antigen in reality constitutes a volumetric ratio of about 8:1, inasmuch as the surface tension of the antigen is only one-half of that of the serum.

Examination: The slide is examined under the low power microscope (magnification 1:80), the diaphragm being sufficiently narrowed. The reaction is clear cut. In negative sera the whole field is uniformly bluish and has a fine granular appearance without any clumping. This appearance becomes particularly evident when the lens is focused upon the surface layer. In positive sera a definitely marked clumping is observed. The clumps are stained more intensely than the surrounding fluid. Their size varies. Big clumps indicate a *strongly positive* reaction, clumps of medium size are reported as a *positive* reaction, and fine delicate clumping is reported as a *dubious* reaction (±). The clumping is very characteristic and can easily be distinguished from other particles which may be due to the impurities of the serum, to the incomplete dispersion of the antigen in the serum, or to the presence of precipitated cholesterin crystals.

Specificity: The test was performed on 1066 sera and checked by the Wasserman reaction. The following table gives a comparison of the results obtained.

TABLE I.

	Wasserman	Rosenthal
negative	739	735; 4 posit. with a history of syphilis
++++, +++, ++	228	228
+	58	55 posit.; 3 dubious
(dubious) ±	38	19 posit.; 17 dubious; 2 negative
anticomplementary	3	2 negative; 1 positive

The chart shows almost a perfect agreement of the two tests. It seems that in weak positive and dubious Wasserman sera this test gives a more clear cut reaction. Thus the test combines reliability with technical simplicity.

4603

Possible Water Balance; Effects of Alkaline Anterior Pituitary Extracts.

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In these studies an inbred strain of well-standardized mice were used as experimental animals. Feeding and environmental conditions were made as nearly identical throughout as possible. The experiments continued over a period of 8 months, and included many series of animals injected and handled under a wide variety of conditions, but with each experiment carefully controlled. In each case the group was divided into animals receiving the alkaline extract of the anterior lobe, a group receiving the ammonium sulphate extractive, and a group of controls. In some of the experiments, the controls received an alkaline liver extract, on others, normal saline, while in a few cases no injections were given the controls. In some cases the dosages were very minute and given only once a day; from this, they varied to 3 times a day and very large dosages. In some series, all of the animals received unlimited quantities of fluid—water or milk, or both; in others, the fluid intake was sharply curtailed. This series of studies reveals the following facts:

Immature animals injected with the alkaline extract, as outlined by Evans,[1] gain weight at a more rapid rate than do the ammonium sulphate extractive injected animals or the controls, provided the allowance of fluid is unlimited or large. The animals receiving the ammonium sulphate extractive gain weight slightly more rapidly than do the control animals. If the fluid intake be sharply curtailed, the animals receiving the alkaline extract do not gain weight as rapidly as do the control animals, or the ammonium sulphate animals. Under these conditions, however, the ammonium sulphate injected

[1] Evans, H. M., Harvey Lectures, 1923-24, 212.

animals gain weight more rapidly than do the controls. If, after a long-continued period of injections, when the alkaline extract injected animals have gained a great amount in weight, the fluid intake be suddenly stopped, their weights will drop to near the average weight of the control animals in a period of 24 to 48 hours. This result was so peculiar that it was deemed wise to analyze the content of the tissues of the experimental animals. For this purpose, each group was ground and thoroughly mixed separately, and was then dehydrated and ashed. It was found that the animals receiving the alkaline extract under unlimited fluid intake conditions had from 6% to 8% more water than did either the controls or the ammonium sulphate injected animals, the latter two groups of which were very close together in their analyses. It was also found that the ash content of the alkaline extract injected animals was about 3% less than the ammonium sulphate injected, or the control animals.

It is believed that these results indicate the presence of a water balance principle in the alkaline extractive of the anterior lobe of the pituitary.

4604

The Presence of Nerve Fibres in the Dentinal Tubules of Mammalian Teeth.

WILLIAM G. DOWNS, JR., AND CHARLES MAYO GOSS.
(Introduced by Raymond Hussey.)

From the Departments of Pathology and Anatomy, Yale Medical School, New Haven, Connecticut.

For these studies, a number of different forms and methods of preparation were employed. The teeth used were from human, dog, cat, and rodent. Methods utilized included supra- and intra-vital methylene blue, various modifications of the Cajal technique, and iron hematoxylin preparations, following in large part the various studies on this subject by other workers. By only one method was it possible to stain the structures in such a way as to make the evidence incontrovertible. By means of pyridine fixation, the Cajal technique of silver impregnation, and very careful grinding instead of decalcification, it was possible to so prepare the mammalian tooth as to show adequate evidence that the dentinal tubules contained definite, unmyelinated fibers. These could be traced in separate portions of the same preparation from their arborizations around the odontoblast, extending thence into the dentinal tubules, following these

tubes to the dento-enamel junction, where they apparently dichotomize between the dentin and enamel. By careful grinding technique, it was found to be possible to prepare sections by this method sufficiently thin that the oil immersion lens with very high power magnification could be used.

4605

High Frequency Current Burns in Rats.

W. M. BALDWIN AND M. DONDALE.

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In a recent paper[1] attention was called to destructive histologic results produced in the small intestine of adult albino rats through the agency of high frequency currents. Certain comparisons were possible between these results and the type of duodenal ulceration observed in man and thought at one time to be a sequence of cutaneous burns.[2] The ulceration produced by high frequency currents was more extensive, however, and not restricted to the duodenum. Yet, Stengel[3] has called attention to the occasional presence of ulceration in the stomach and elsewhere in the intestine, complicating burns of the skin in man.

Certain experiments upon adult albino rats were repeated, utilizing high frequency current as detailed in the previous paper. Particular attention was given, however, to the problem of mucosal regeneration of the intestine. Repeated heatings on alternate days with the rats parallel to and between the plate electrodes, which were spaced 19 cm., gave the most constant histologic changes. The amperage remained 0.2 and the voltage 2000. The rat temperatures reached 41°C. after exposure of from one-half to 2 hours of raying.

The apices of the villi demonstrated the first pathologic change. This was of the nature of a coagulation necrosis and was most marked, first, in the epithelial lining cells but involved the stroma cells ultimately. Exfoliation of the epithelial cells was the rule, while vascular dilatation, leucocytosis, hemorrhages into the villi,

[1] Baldwin, W. M., and Nelson, W. C., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 588.

[2] Novak, E., *Am. J. Med. Sc.*, 1925, clxix, 119.

[3] Stengel, A., *Osler's Modern Medicine*, Lea & Febiger, Philadelphia and New York, 1908, v, 389.

submucosa and muscularis were the characteristic changes encountered. Pyknotic nuclei with fragmentation and degeneration of both nuclei and cells were likewise often encountered in the mucosa and submucosa, especially in the intestinal and duodenal glands. These microscopic features ordinarily extended, when the burning was severe, throughout the entire length of the small intestine. They have been observed, further, in the pyloric end of the stomach and throughout the large intestine as far as the rectum. The gut tube was filled with tissue detritus.

The regenerative process was begun soon after the last raying and in most instances was completed by the fifth or seventh day. The vascular dilatation subsided, the leucocytosis disappeared and the fragmented and degenerated cells were replaced by the mitotic process. The gland cells exhibited active mitosis. The most striking features were observable, however, in the epithelial cells covering the villi. Such cells were replaced by active mitosis of the epithelial cells remaining uninjured in the depths between adjacent villi. This regenerative process extended, therefore, from the base to the apex of the villi. At first these cells were flattened, but gradually they assumed short columnar and lastly long columnar proportions. The cuticular free margin was the last to be replaced. Ultimately the gut wall was restored to normal histologic appearance. There was no scar formation.

However, instances were observed where this repair process was delayed over as much as 2 months. In these cases the villi persisted as short stubby outgrowths of the mucosa with very much shortened long axes and often inclined at an angle and matted together so that the line of demarcation between adjacent villi was rendered indistinct as seen microscopically. This villous mass, apparently effected through the coalescence of adjacent villi, was covered with a single layer of short or almost flattened epithelial cells lacking a cuticular margin but directly continuous with the normal epithelial cells of adjacent intact villi. Positive evidence could not be obtained that such cells were at any time derived from metamorphosed stroma cells of the villi. Apparently, such villi functioned normally, to an extent, at least, since the rats were normal in health and in reaction.

4606

Origin and Proliferation of Thrombocytes in Splenectomized Salamanders.

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In normal salamanders *(Triturus viridescens)* thrombocytes are differentiated chiefly in the spleen. In splenectomized salamanders that were kept in excellent condition for one year after total extirpation of the spleen, thrombocytes were noted in all stages of development in the general circulation. The blood smears made from certain of these animals and stained with Wright's stain have afforded exceptionally favorable material for the study of thrombocytopoiesis.

The question of the origin of the thrombocytes has been at issue for many years. There is little unanimity of opinion concerning the type of cell from which the thrombocyte is derived, or whether its locus of differentiation is intra- or extra-vascular. Among the later investigators may be mentioned Sugiyama,[1] who holds that the thrombocyte is a derivative of the megaloblast, a hemoglobin-containing cell; Gordon,[2] that it is a senile erythrocyte; Maximow,[3] that it is a derivative of the lymphocyte; Hartmann,[4] that it is a cell genetically and structurally comparable to the megacaryocyte of mammals (as suggested by Wright[5]) which furthermore differentiates in the bone marrow in extravascular location only; Jordan and Speidel,[6] that it is a derivative of the small lymphocyte which has a minimum of cytoplasm.

Our observations on splenectomized salamanders, however, point unmistakably to the large lymphoid hemoblast (hemocytoblast) as the ancestral cell. In our blood smears prepared according to Wright's technic, the young thromboblasts are easily distinguishable from the young cells of the erythrocyte series. The thromboblasts present a characteristic reddish or reddish-violet fine granulation in the cytoplasm, quite different from the color shades of the proeryth-

[1] Sugiyama, S., Carnegie Inst. of Wash., Pub. 363, Contrib. to *Embryol.*, 1926, xviii, 121.

[2] Gordon, L., *Virchow's Arch. f. Path.*, 1926, cclxii, 19.

[3] Maximow, A., *Arch. f. mikr. Anat.*, 1923, xcvii, 623.

[4] Hartmann, E., *Fol. haematol. Arch.*, 1925, xxxii, 1.

[5] Wright, J. H., *J. Morph.*, 1910, xxi, 263.

[6] Jordan, H. E., and Speidel, C. C., *Am. J. Anat.*, 1929, xliii, 77.

roblasts, the stages between the hemoblast phase and the true erythroblast.

The megaloblast can be eliminated as the progenitor of the thromboblast. Megaloblasts are plentiful but there is no sign of transition stages toward the thrombocyte. No trace of hemoglobin can be seen at any stage in thrombocytopoiesis. Furthermore, in the salamander it is apparent that the thrombocytes cannot be senile or degenerate red cells. Senile red cells occur in large numbers, but all stages in the process of degeneration can readily be distinguished from the cells of the thrombocyte series.

Of special interest and importance is the observation of thromboblasts in mitosis. As far as we can ascertain thromboblasts have not up to this time been seen in mitosis in the circulation, certainly not in adult animals. In fact, they have rarely been seen in mitosis in any location. A small number of unequivocal examples of thromboblast mitosis have been seen by us in blood from different salamanders. These cells in anaphase contain the fine reddish granulation that is quite characteristic of thrombocytes in this species, and are beyond doubt genuine thromboblasts. This observation would seem to cast grave doubt on all theories which regard thrombocytes as degenerate red cells or other types of cells. The thromboblast in salamander appears to be on the same footing as the erythroblast, eosinophilic granuloblast, and neutrophilic granuloblast; all of these being capable of proliferation by mitosis at the stage of development after the appearance of their specific cytoplasmic differentiation.

The plentiful occurrence of young thromboblasts in the circulation, and of various stages in the process of differentiation, would seem to indicate intra-vascular thrombocytopoiesis in this species. The salamander has no bone marrow (the place where extra-vascular thrombocytopoiesis is described by Hartmann in the toad) and in our experiments was deprived of the spleen. In the lympho-granulocytopoietic capsule of the liver, which is partly analogous to the bone marrow of higher forms, there was no sign of extra-vascular thrombocytopoiesis.

4607

Pathological Changes in the Viscosity of Blood Serum.

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Measurements of the viscosity of blood serum reported by clinical observers have shown results that are seemingly paradoxical. We have attempted to investigate the physical-chemical basis of these empirical relationships.

Blood serum, as seen from the viscosity at different dilutions, shows a remarkable ability to keep its viscosity constant in spite of dilution. If we compare the effects of dilution on the viscosity of such a protein as gelatine, we find that not alone is the absolute viscosity of the same percentage concentration of serum protein very much lower than that of a similar strength of gelatin, but the gelatin manifests a much greater change in viscosity for each increasing unit of dilution. The importance of this point in maintaining the circulation of the blood in such conditions as nephrosis, where the blood proteins are reduced to one half their normal concentration, is obvious.

Loeb has distinguished between two types of protein, one represented by gelatin and the other by egg albumin. In the former there is an enormous tendency toward the formation of submicroscopic particles of solid gelatin which, as a result of the inability of the protein ion to freely diffuse, set up a Donnan equilibrium with the result that water is occluded and these particles increase in size. On the other hand, we have such proteins as albumin, which show no tendency at a pH in the neighborhood of neutrality and at body temperature toward the formation of these submicroscopic particles. These show a low viscosity and no tendency to jell. There are certain exudates, especially pleural exudates, which after standing become jellified. If Loeb's hypothesis were correct, these exudates should show a higher viscosity than other exudates and transudates that do not jell. We have found that abdominal exudates of higher protein concentration that did not jell had a lower viscosity than some other exudates of lower protein concentration that did jell. This would seem to prove Loeb's theory of the presence of submicroscopic particles, in this case probably of fibrin, which cause the solution to manifest higher absolute viscosity and which make it occlude water.

Those proteins which show no tendency to jell also show very

little effect on the viscosity of their solutions through changes in the hydrogen ion concentration. The viscosity of blood proteins is very insensitive to changes in the hydrogen ion concentration, the viscosity remaining almost constant between the ranges of pH 28 and 81.

In an effort to explain the high viscosity found in the serum of patients suffering from cyanosis, CO_2 was passed through blood serum. After that the cells were put back with the serum and the CO_2 passed through again. After centrifuging, the viscosity of the serum was again measured and was found to have increased. Von Limbeck shows that saturating the blood with CO_2 causes the cells to swell up with water which passes into them from the serum, and it is for this reason we find that the viscosity of serum containing cells through which CO_2 has been passed is increased.

In cases of nephritis we should expect that owing to the increase in total solids such as urea, cholesterol, etc., the viscosity of the blood serum would be much increased. Such, however, is not the case. The figures for the viscosity of blood serum of patients suffering from nephritis have always been found normal. We have found that the saturation of serum with cholesterol reduces its viscosity. This is probably due to the tendency of hydrophobe colloids such as cholesterol to emulsify water in oil in contradistinction to the hydrophile colloids such as albumin, globulin, lecithin, etc., which tend to emulsify oil in water. W/O emulsifiers increase the size of the fat particles and cause a fall in the viscosity.

It has been known for a number of years that the effect of urea has been to decrease the viscosity of water. We have found that the viscosity of blood serum is similarly decreased by the addition of urea. It is of some significance that just those blood constituents, such as cholesterol and urea, which are found higher as a result of kidney lesions, have the power of lowering the serum viscosity. In about 5 cases of uremia, all of which showed abnormally high urea and cholesterol figures, we found, again in agreement with findings of clinical investigators, that the viscosity was fairly normal.

The serum of patients suffering with pernicious anemia and hemolytic jaundice, though showing an increase in the concentration of bilirubin, does not have a higher viscosity. On the other hand, sera of patients suffering with obstructive jaundice show an enormous increase. The presence of bile acid in cases of obstructive jaundice would seem to offer an explanation for the difference in the viscosities of the sera of patients suffering with these diseases. The bile acids are known to greatly decrease the surface tension and increase the viscosity. We should, therefore, expect

that a blood serum showing a positive direct Van den Bergh reaction will have a much higher viscosity than one showing only a positive indirect. Such we found to be the case.

4608

Relationship Between Vitamin C and Oestrus in the Guinea Pig and the Fertilizing Power of Sperm.*

MARIANNE GOETTSCH. (Introduced by G. L. Foster.)

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Papanicolaou and Stockard[1] have shown that dioestrus is prolonged when guinea pigs are underfed. The effect of vitamin C deprivation was studied by their method in the following way.

After their oestrus rhythm had been observed on a complete natural food diet, 3 guinea pigs were transferred to the Sherman scorbutic diet with a daily protective dose (3 cc.) of orange juice. The regular occurrence of oestrus was soon resumed and maintained for about 150 days, during which the orange juice was fed successively at the levels of 3 cc. ½ cc., and ¼ cc. daily for about 50 days on each level. Two of the pigs manifested oestrus after the level of orange juice had been reduced to ⅛ cc., just before the rapid decline in growth, due to scurvy, set in. Two of the pigs were autopsied within 20 days of the last cycle and showed large follicles as well as the marked symptoms of scurvy. The third pig was cured by the administration of 3 cc. of orange juice daily, and the ovulation rhythm was reestablished within 10 days of the rise in the growth curve.

Daily weighings indicated that during oestrus, the guinea pig lost from 30 to 40 gm. in weight and regained it shortly thereafter. There was no corresponding decrease in food consumption, such as Slonaker[2] has observed in the rat.

Lindsay and Medes[3] found that guinea pigs with mild chronic scurvy did not reproduce, and they described extensive histological

* This work has been conducted with the aid of the departmental Research Fund of the Chemical Foundation.

[1] Papanicolaou, G. N., and Stockard, C. R., PROC. SOC. EXP. BIOL. AND MED., 1919, xvii, 143.

[2] Slonaker, J. R., *Am. J. Phys.*, 1924-1925, lxxi, 362.

[3] Lindsay, B., and Medes, G., *Am. J. Anat.*, 1926, xxxvii, 213.

changes in the testes. In the present experiments, however, motile sperm were found in the epididymis of males dying from scurvy. In order to acquire physiological proof of fertility, functional tests were initiated in which 6 adult males were gradually deprived of vitamin C and mated at intervals with normal females in oestrus. Only one attempted mating of 43 produced a litter and that one occurred while the male was receiving 3 cc. of orange juice a day. Artificial insemination was therefore resorted to. When sperm from a male, dying of prolonged scurvy, was injected into the uterine horns of a female in oestrus, a litter of normal young, which are now 6 months of age, was born.

Deprivation of vitamin C does not disturb the oestrus rhythm of the guinea pig until the animal begins losing in weight, nor does it interfere with the fertilizing power of sperm.

4609

Immunologically Symmetrical Proteolysis.

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If 0.1 cc. horse serum is added to 1.9 cc. canine leucocytic extract and the mixture is incubated over night, titration of the resulting lytic products by means of ice-chest ripened rabbit precipitin gives precipitin graphs[1] suggesting a 400% to 800% test-tube multiplication of horse proteins, without appreciable horse protein denaturization.

The simplest explanation of this apparent increase is to assume that under the influence of leucocytic proteolysin each horse protein molecule is hydrolysed into from 4 to 8 daughter protein molecules, each daughter molecule being of approximate horse protein specificity.

[1] For technic and typical graphs see PROC. SOC. EXP. BIOL. AND MED., 1929, xxvii, 14.

4610

"A Method for Assay of Ovarian Hormone in Blood and Urine and Relation of Assay to Menstrual Cycle."

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From the Laboratories of Mt. Sinai Hospital, New York City.

A communication with the above title, by F. F. Wildebush and J. F. McClendon[1] describes a technic for extracting the female sex hormone ("ovarian hormone") from the blood and urine by means of which they obtained a minimum yield of 24 M.U. and a maximum yield of 126 M.U. for 20 cc. from the vein blood of normal women. Their method varies from the technic published and employed by us since 1925, mainly in that they add N sodium hydrate to the oxalated blood before extraction with ether.

In our early work we employed oxalated blood, but as routine have long since used anhydrous sodium sulphate to dry the blood and performed a dry extraction. Our work in 1925 had convinced us that the dry is preferable to the wet extraction.

In over 550 women on whom we have performed more than 1000 tests, our readings have never shown a greater yield than *1 M.U. in 40 cc. of vein blood of normal women* except during pregnancy, and then not above 2 M.U. have been found. The lowest readings of Wildebush and McClendon are therefore *50 times as great as ours; their highest 252 times as great.* On how many women their figures are based cannot be gathered from their report.

We have attempted to elucidate the cause for this extreme and startling divergence by repeating their work, duplicating their technic with scrupulous exactitude. The amount of the extract given to castrated mice varied when based upon Wildebush and McClendon's minimum maximum results, from a possible 0.6 to 6 M.U. as a minimum, up to 3 to 31.5 M.U. as a maximum.

Our results are based upon bloods of 4 patients, the extracts injected into 16 mice. Not a single positive reaction was obtained.

We have not yet had the opportunity to repeat their work on urines. We make no attempt to explain the fact that our results with the Wildebush and McClendon technic are uniformly negative. We desire, however, to call attention to the fact that unless the oxalation and alkalinization of blood serum *releases female sex hormone contained in an inactive state in the blood serum,* the injection of

[1] Wildebush, F. F., and McClendon, J. F., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 785.

0.1 to 0.5 cc. of untreated blood serum in castrated mice (granted that Wildebush and McClendon's observations are correct) should give a positive reaction. Yet the results of many investigators have uniformly shown that even 10 to 15 cc. of untreated blood serum of normal non-pregnant women, produce no reaction.

TABLE I.

Days of Cycle	Amounts of extract used in each case equivalent to	Mouse units to be expected according to Wildebush and Mc Clendon*	Mouse units found
27th day after menses	1/2 of 10 cc. blood	30-31.5	0
26th day after menses	1/5 of 10 cc. blood	12-12.6	0
24th day after menses	1/10 of 10 cc. blood	6- 6.3	0
23rd day after menses	1/20 of 10 cc. blood	3- 3.1	0

* At this period of the cycle.

4611

The Development of Movement of the Hind Leg of Amblystoma.

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The earliest movements of the hind leg are adduction and abduction, and they occur only with action of the trunk. At about the same time that this type of movement appears, or probably a little later, the hind leg elevates when the animal is rotated dorsally on its longitudinal axis on the side of the reacting limb. This movement of the hind leg is co-ordinated with elevation of the fore limb. This integrated action of fore and hind limbs with action of the trunk is a typical postural reaction and it occurs before a local reflex of the hind leg can be excited. Before local exteroceptive reflexes of the hind leg appear there occur also strong simultaneous abduction of both hind legs in coordination with elevation of the head and fore part of the trunk; the typical walking posture, *i. e.*, flexure of the trunk with the adduction of the fore legs and abduction of the hind leg on the concave side while on the opposite side the fore leg is abducted and the hind leg adducted; and, at least in many cases, typical walking.

Tactile stimulation of the leg excites action of the animal as a whole until just before local reflexes of the leg appear, when such stimulation inhibits all body movement. Local reflexes of the hind

leg in response to exteroceptive stimulation on the leg and on the skin of the trunk behind and near the leg make their appearance at about the same time. These reflexes begin at about the same time that antigravity action of the legs can first be detected. This is before there is rotation of the leg or passive bending of the knee under antigravity pressure.

The leg begins to rotate passively under antigravity pressure at about the time that antigravity action of the leg can first be detected and this is before there is flexion of the knee; but antigravity action of the leg has been observed in specimens in which there was no passive rotation of the leg.

The plantar reflex begins as an action of the leg as a whole, and only later is restricted to action of the distal segments of the leg. At about the time that active flexion of the knee appears in walking there is the first reflex flexion of the knee, foot and digits in response to plantar stimulation, and active extension of the knee in walking appears at about the same time. Stepping backward first appears after the plantar reflex stage of the digits, and in the earliest observed case of the backward step there was active extension of the toes as the animal lifted the foot from the substratum in the extreme position of abduction.

Active rotation of the leg in walking makes its appearance distinctly after there is passive rotation of the leg under antigravity pressure and it first occurs in the extreme phase of abduction.

4612

Accumulation of Potassium in Living Cells—a Non-equilibrium Condition.

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Previous workers[1, 2] have suggested that the relative excess of potassium observed in divers living cells and tissues might be accounted for by assuming that ionic equilibrium is not attained during the life of such systems but is most nearly approached by the most mobile ions. But no completely satisfactory theory has yet been proposed, nor has any experimental proof been adduced. This

[1] Andrè, G., and Demoussy, E., *Bull. Soc. Chim. Biol.*, 1925, vii, 806.

[2] Osterhout, W. J. V., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiv, 234.

paper gives experimental proof that a non-equilibrium state exists, and explains the mechanism of accumulation.

If in its normal state a living cell were in ionic equilibrium with the surrounding medium any increase in the concentration, or, more properly, in the activity of an ion in the surrounding medium should, if it leads to any change, result in an increase in the activity of the same ion within the cell. Conversely, decrease outside should lead to decrease inside. If decrease outside should be found to produce increase inside or vice versa, it appears impossible to account for the observed facts except by assuming the existence of a non-equilibrium state.

Experiments were made on the unicellular coenocytic marine alga *Valonia macrophysa* Kütz. from which large amounts of intracellular sap may be obtained for analysis. Under normal conditions this sap has a pH of about 6.2 and potassium and sodium ion concentrations of roughly 0.5 M and 0.1 M, as compared with a pH of 8.2, and potassium and sodium ion concentrations of roughly 0.01 M and 0.5 M, respectively, in sea water. Since both this sap and sea water are dilute aqueous solutions containing little or no organic material, no sensible error will be introduced by considering ion concentrations rather than activities.

Sufficient isotonic sodium chloride solution was added to different samples of sea water to reduce the potassium ion concentration to 90, 75 or 50% of that in normal sea water. Comparable lots of about 60 *Valonia* plants were placed in these solutions for different periods varying from 9 hours to 8 days, with control lots in normal sea water. After exposure, the sap was collected, and the concentration of sodium, potassium and chloride determined in each. These analyses show that reduction in the potassium content of the sea water leads to a slight but distinct increase in its concentration in the sap. This can only be accounted for by assuming that the cells are not normally in ionic equilibrium with the surrounding sea water.

The way in which this non-equilibrium state is maintained may be pictured as a result of a mosaic constitution of the protoplasmic surface. Areas permeable to cations only alternate with areas permeable to anions only, the size of the areas being somewhat greater than the effective diameter of the electrical fields surrounding the ions present.[3, 4] In the normal state carbonic acid produced by protoplasmic metabolism will dissociate yielding hydrogen and bicarbo-

[3] Michaelis, L., *J. Gen. Physiol.*, 1925, viii, 33.

[4] Höber, R., and Höber, J., *Arch. ges. Physiol.*, 1928, ccxix, 260.

nate ions. The hydrogen ions thus produced inside the cell reach a concentration of about 100 times that in the surrounding sea water, as can be shown by direct measurements, and will therefore pass out through the cation permeable areas, other cations entering the cell through the same areas to maintain local electrical neutrality. In this process the available cations will enter at rates determined by the combined effects of their activity gradients and their penetrabilities through the protoplasmic surface. By analogy with the cation permeable "dried" celloidin membrane we may expect these penetrabilities to follow the same sequence as the mobilities of the different ions in free diffusion, but to differ much more widely from ion to ion. Under these conditions penetrability will outweigh activity gradient and therefore more potassium will enter the cell than sodium. The divalent cations (Ca and Mg) will also penetrate slowly. The bicarbonate ion like the H ion will be present in the sap in a concentration many times exceeding that in the surrounding sea water, and will pass out through the anion permeable areas, its place being taken by chloride in order to maintain local electrical neutrality. Since these processes lead to a continued increase in the amount of osmotically active material in the cell, the cell must take in water and grow.[2] In this way attainment of equilibrium is prevented so long as CO_2 is being produced by the cells, and potassium accumulates in the cell.

Exposure of *Valonia* cells to sea water mixed with isotonic sodium chloride may be supposed to increase the disparity between the penetrabilities of potassium and sodium and hence lead to the observed increase in the proportion of potassium. Presumably any slight increase in permeability, no matter how caused, would lead to the same result. We may picture the protoplasmic surface as being of a porous nature, the pores being capable of variation in diameter, and owing their restricted ion permeability to charges resident in the walls of the pores. Hence these ions with the smallest effective diameters (as judged by their mobility in dilute aqueous solution) will penetrate most readily, and for each ion there will be a characteristic relation between pore diameter and penetrability. We may suppose this to take the form shown in Fig. 1 for different cations. In the case of *Valonia* the normal state of the cell would be characterized by the relative penetrabilities shown at A; in our experiments the state would be like that shown at B, the penetrability of $K^{\cdot}$ being increased relative to $H^{\cdot}$ and $Na^{\cdot}$. Serious injury or death would lead toward the condition shown at C. It is assumed that $HCO_3^{\prime}$ and $Cl^{\prime}$ pass through their appropriate type of area

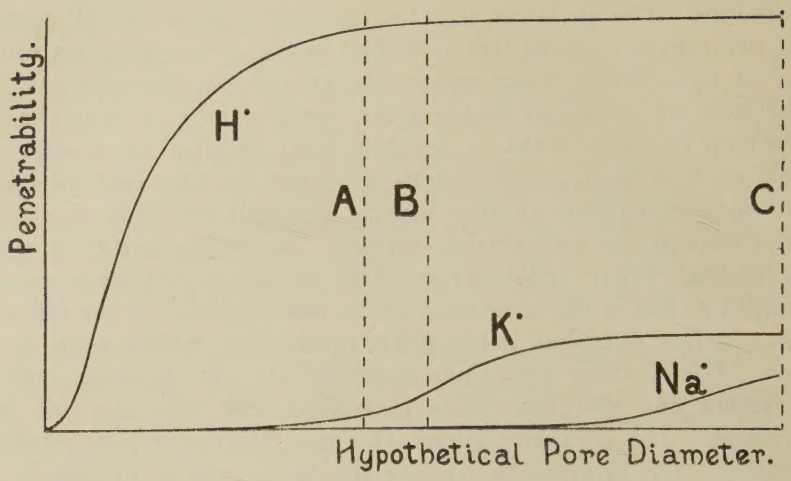

FIG. 1.

with relative ease. The intake of many substances, especially organic compounds, may be complicated by factors other than those here considered.

Thus we may account for such diverse phenomena as the intake of ions by vascular plants, the accumulation of vanadium in the blood of *Ascidia,* the different ratios of potassium to sodium in mammalian erythrocytes, and so forth. Physiological alterations in the end products of oxidation, (*e. g.,* lactic acid production) or in protoplasmic permeability should lead to corresponding alterations in the ion content of cells and tissues such as are known to characterize plant galls and malignant tumors in animals.